IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Geiss et al.

Application No.: 10/695,427

Filed: 10/28/2003

Title: Method of Treating Extreme Physical or Mental Stress Using L-Theanine to Obtain Accelerated Regeneration

Confirmation No. 4477

Attorney Docket No: 7390-X03-020

Group Art Unit: 1614

Examiner: Spivak, Phylis G.

DECLARATION UNDER RULE 1.132

I, Prof. Michael Weiss, declare, based on his personal knowledge, the following:

1. That I am one of the inventors of U.S. Patent Appl. Ser. No. 10/28/2003 titled, "Method of Treating Extreme Physical or Mental Stress Using L-Theanine to Obtain Accelerated Regeneration", and am familiar with the contents of the application and the claims.

2. That I am a professor at the University of Paderborn, Paderborn, Germany.

3. That there is an Institute for Sports Medicine at the University of Paderborn.

4. That in conjunction with and under the sponsorship of the ISME GmbH, Moerfelden-Walldorf, Germany, I and my colleagues at the University of Paderborn designed and carried out a clinical study to determine the effects of L-Theanine on the recovery and regeneration of persons subjected to extreme physical stressing at the Institute for Sports Medicine at the University of Paderborn.

5. That the study noted in para 4 was carried out under my supervision and control on behalf of myself and my co-inventors, and completed no later than December, 2000.

6. That the clinical design and clinical methods, the participants in the clinical study and the clinical results and data of the study including 35 tables and 15 figures are detailed in a FINAL REPORT ABOUT THE SCIENTIFIC STUDY ON L-THEANINE CONTAINING DRINKS WITH REGARD ON RELAXATION AND REGENERATION AFTER PHYSICAL STRESS AS MEASURED BY ELECTROENCEPHALOGRAPHY, SKIN CONDUCTANCE AND STRESS HORMONES, copy attached, of which I am the principal author and editor and was

directly responsible for, supervised and determined its content.

7. That, as described in the FINAL REPORT, 15 subjects (students) were initially in the study, but one dropped out. The sample size of the study was resolved to fourteen (14) test subjects. The remaining 14 subjects were tested while connected for electroencephalography (EEG) recordings, skin conductance measurements, periodic blood tests and urine tests, heart-rate measurements, and blood pressure measurements. As a first step, they were then exercised to near physical functional capacity. As a second step, they were then given beverages to drink that contained one of a placebo, 50 mg of L-theanine, and 200 mg of L-theanine, as a third step, they were then made to lie down to regenerate, and as a fourth step, measurements were taken at specific time periods and recorded during their recovery and regeneration to a rested or relaxed condition or state.

8. That to achieve the state of extreme physical stress in the first step, an ergometer (i.e. a stationary bicycle) was used. The subjects exercised with an increasing load (stressing) over sixteen (16) minutes until nearly reaching exhaustion. After the subjects stopped riding the stationary bicycles, in step two they were given a drink containing one of a placebo, 50 mg of L-theanine, or 200 mg of L-theanine, as noted above, and their recovery and regeneration was then monitored in steps three and four for the parameters noted above, at specific time intervals as shown in Fig. 1 of the Report, designated as M1 - Start of monitoring (extreme physical stress) within 1 minute of conclusion of stressing; M2 - 30 minutes after M1 (a transition period between extreme physical stress and drowsiness); M3 - 45 minutes after M1 (drowsiness period); M4 - 60 minutes after M1 (a transition period between drowsiness and regeneration; and M5 - 120 minutes after M1 corresponding to end of regeneration, see further details in the Report. The time interval designations M1 to M5 are shown in the patent application, as noted above, with correlation to graphic portrayals reflecting the condition of the brain at the respective time intervals.

9. That the effect of the exercising at near physical functional capacity was evidenced by an increase in heart rate. The near-maximum exercise produced a stress reaction in the subjects, which was determined as being severe after examining their blood chemistry. Blood samples were taken immediately after the exercise. The examined blood showed an increase in the numbers of leukocytes, blood glucose, catecholamine, and serotonin. Samples were also taken at M2 and M3, 44 minutes and 59 minutes after exercise, and showed elevated concentrations of prolactin and cortisole.

10. That the subjects taking the placebo evidenced a full recovery and regeneration of all blood values and reached a relaxed and rested condition and state in about two (2) hours.

11. That the subjects taking drinks containing L-theanine evidenced an accelerated full recovery and regeneration of all blood values and reached a relaxed and rested condition and state in about thirty (30) minutes.

12. That in addition to the foregoing:

a) no statistically significant difference in levels were observed of epinephrine, norepinephrine, dopamine, and serotonin;

b) a difference in hormone levels of prolactin and hematocrit were observed in subjects drinking L-theanine;

c) a difference in EEG results was observed in subjects treated with L-theanine, but the difference was not general, but localized in areas of the brain and only at specific frequencies;

d) no change in electrosympathography was observed in patients consuming L-theanine containing drinks;

e) L-theanine seemed not to influence receptor adaptation or regulation based on measuring CyclicAMP; and

f) no difference in urine samples was observed from measurements of metabolites like creatine in subjects treated with L-theanine.

13. That based on the clinical data collected and analyzed, as shown and reported in the Report noted above, L-theanine did not influence the regulating function of central-nervous system hormones or the excretion of their metabolites, nor did it influence brain activity in a serious manner (although changes in some alpha and beta waves were observed, which is compatible with prior studies regarding L-theanine and relaxation). Despite the absence in change in central-nervous system hormone levels, the levels of prolactin (which have been shown in the known prior art to be controlled by those hormone levels) was affected. This led to the conclusion that regeneration and recovery from extreme physical stressing was due to a coupling between the central nervous system and the peripheral endocrine system influenced by L-theanine.

14. That acceleration in recovery and regeneration after extreme physical stress to about 30 minutes was due to heightened serum prolactin levels, as well as correlations between EEG-parameters and peripheral hormone concentrations resulting from the ingestion or consuming of 50 mg to 200 mg of L-theanine with indications that L-theanine influences the coupling between central nervous controls and peripheral controls, see Report.

15.That based on the activity performed and the clinical data collected during the study noted above and reported in the Report titled, "Final Report about the Scientific Study on L-theanine Containing Drinks with Regard on Relaxation and Regeneration after Physical Stress as Measured by Electroencephalography, Skin Conductance, and Stress Hormones." noted in para 6 above, the invention as described in the patent application noted in para 1 above, and now claimed, was not only actually reduced to practice, but the utility and efficacy of the invention was established.

16. That further, the activity performed and the clinical data collected during the study and contained in the Report evidence and corroborate the actual reduction to practice and the utility and efficacy of the invention regarding accomplishment of its intended purpose, namely, to accelerate recovery and regeneration of a human experiencing extreme physical stressing to a relaxed and rested condition and state.

17. That still further, the activity performed and the clinical data collected during the study, evidence, prove and demonstrate the novelty and unobviousness of the claimed method for accelerating recovery of humans experiencing extreme physical stress to near functional capacity comprising feeding a human experiencing extreme physical stress near physical functional capacity 50mg to 200 mg of L-theanine mixed in a foodstuff or drink, and resting the human, following consumption of the mixed foodstuff or drink, for a period of at least 30 minutes whereby the recovery and regeneration to a relaxed condition is accelerated.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Prof. Michael Weiss

dated: 2007/03/26

Final Report
about the scientific study on L-theanine containing drinks with regard on relaxation and regeneration after physical stress as measured by electroencephalography, skin conductance and stress hormones

M. Weiss, Th. Barthel, R. Schnittger, C. Reinsberger
Institute for SportsMedicine
University of Paderborn, Germany
By order of
ISME GmbH, Mörfelden-Walldorf, Germany

Introduction

From the up to date literature it can be derived that L-theanine has no pharmacological effects like a tranquilizer nor does it act like a neurotransmitter antagonist. In animal studies it seemed to interact with the pathways of distinct neurotransmitter systems, i.e. the dopaminergic, serotoninergic and the noradrenergic system in the brain at the pre- and post-receptor level (concentrations and release of the transmitters and the norepinephrine stimulation of the second messenger cAMP). In human beings it was shown that L-theanine influences the cerebral electrophysiological activation (EEG alpha-waves). Derived from literature and published individual observations and since dopamine and norepinephrine are part of the stress system the study was based on the hypothesis that L-theanine has relaxing effects and influences stress management and coping. To objectify this at an endocrinological and electrophysiological basis we used topographical frequency spectrum electroencephalography-measurements (EEG-mapping), skin conductance measurements (electrosympathography, ESG), and determined blood stress hormone levels after exercise stress under standardized conditions as well as the basal and stimulated cAMP production of blood mononuclear lymphocytes (MNL) taken in the regeneration phase after stress. Bycicle ergometer tests were used as a reliable, very well reproducable and individually adjustable stress model.

Study design and methodes

Probands: The study started with n=15 nonsmoking male healthy students of physical education who were accustomed to heavy physical exercise but not specially trained in endurance sports or weight lifting. They were free of drugs and/or stimulants and gave their written informed consent. By a pretest their integer circulatory adaptation to exercise and their individual working capacity was determined and they were familiarized with the test conditions. One test person dropped out because of illness. For anthropometric and ergometric test data of the 14 test persons who finished the study see table 1. Their maximal performance was 357+/-47 Watt = 4.56+/-0.6 Watt per kg body mass and their maximal heart frequency was 186+/-9 S/min.

The *pretest* was carried out on the same bycicle ergometer like the test trials in upright position, starting at 50 Watt and increasing workload by 50 Watt every 3 minutes until exhaustion. The final *test load* was individually standardized as a step test with 5 equal increments between 0 Watt and the step load that could be maintained for 3 min in the pretest. Steps 1 - 4 lasted 3 min each and the last step 4 min (total stress test time = 16min, see fig. 1 right upper level).

The *test drinks* had identic colour and taste but differed in the L-theanine content: 0 (placebo, *drink 1*) or 50 (*drink 2*) or 200 mg (*drink 3*) of L.theanine in 0,33 l.

The *tests* were carried out in intervals of one week in the morning at the same time (8.00 a.m., 8.35 a.m., or 10.20 a.m. for different groups) and the drinks were given in a randomized cross over double blind order (list of randomization see table 2). In the time between the tests the probands should not change the usual activities and the habits of eating which are protocolled in a diary. The day before each test no intensive exercise nor excesses in drinking or eating were allowed and the probands ensured enough sleeping time. The probands met the institute in the postabsorptive phase one hour before the tests and had a standardized german breakfast with 395 kcal (bread, butter, jam; 50 g carbohydrates, 18.4 g fat, 7.6 g proteine).

mineral water and/or fruit tea ad libitum but without caffeine or other stimulants. After fixing an indwelling catheter in a forearm vein, applicating the electrode caps and testing the electrical resistance of the electrodes the experiments run through like shown in figure 1:

- Incremental stress test on the bicycle ergometer (fig.4, 5, 6)
- Within 1 min thereafter measurement M 1 *a* and *b*
- Ingestion of the drink

Then time started for the following measurements after end of drinking (start of exercise):

- 30 (60) min: M 2 *a*
- 45 (75) min: M 3 *a*
- 60 (90) min: M 4 *a* and *b*
- 120 (150)min: M 5 *a* ,*b* and *c*

During this time probands regenerated from exercise stress passively lying in a segregated shaded room (fig.5) which was connected with the apparatus by cables. So test persons were not influenced by the staff during the measurement procedures (fig. 5 and 6).

Measurements were done in the sequence: blood collection, EEG and Electrosympathograhy (ESG) recording for 3 min, blood pressure measurement (RR). Heart rates were recorded continuously with the Polar® pulse tester. Measurements *a* included the blood parameters [blood cells, hormone levels (epinephrine, norepinephrine, dopamine, serotonine, cortisole, prolactine) and blood glucose concentrations], EEG and RR. Measurement *b* included sampling and isolation of blood mononuclear lymphocytes and measuring their basal and Isoproterenol stimulated cAMP-production. For measurement *c* urine bladder was emptied after ingestion of the drink, then all urine was collected for 2 hours.

EEG-recordings were carried out in the lying position with eyes closed for 3 min. Topographical spectrum analysis and statistic maps were carried out with the CATEEM®system (MediSyst, Linden, Germany) as shown in figure2. For standardized positioning of the 17 tin-electrodes according to the international 10-20 system a electrode cap (Electrode Cap Com, Eaton, Ohio, USA) was used, which was applicated with a special electrode gel (Spektra 360 Electrode Gel, Parker, New Yersey, USA) and tested for correct recording and conductance resistance before the exercise test and was worn up to the end of all measurements.

For the *skin conductance measurements* we used the Electro-Sympathograph ESG X from INES in D-33184 Altenbeken, Germany with electrodes as used for ECG measurements placed at the tips of the fingers 2 and 3 of the left hand (fig. 5).

Blood was collected into vacuum tubes after removal of the first heparin-containing 0.5 ml from the venous catheter. The 10ml tubes for separation of mononuclear lymphocytes (MNL) contained 0.5ml sodium-heparin, the 4ml tubes for cell counting K-EDTA, those for measurement of catecholamines (5ml) EGTA and glutathion as antioxidant. Catecholamine tubes were immediately putted in ice cold water and centrifuged at 4°C for 5 min., serume tubes centrifuged after 20 min. Immediately after centrifugation samples were stored at -80°C until determination. All samples of one test person were measured within one assay. Blood and urine parameters were determined with the following methods:

Serotonine, homovanillic acid, 5-hydroxy-indol-acetic acid (5-HIES): HPLC

Catecholamines (epinephrine, norepinephrine, dopamine): Competitive Radioimmunoassay

Cortisole: Competitive chemoluminiszent Immunoassay

Prolactine: Sandwich chemoluminiszent Immunoassay

Creatinine: Photometric-kinetic method from Jaffe

MNL separation, stimulation and cAMP determination: Mononuclear lymphocytes (MNL) were prepared by density-gradient centrifugation (Lymphoprep, Nycomed Pharma Oslo, Norway, 800g, 20 min) and suspended in theophylline buffer at a fixed number of $2x10^6$ cells/ml. Aliquots were incubated for 2 min with 50 mikrol either buffer or buffer containing isoproterenol in final concentrations of 10 and 100 mikroMol. After cell destruction by the

lysing solution, boiling and centrifugation, the cAMP concentration in the supernatant was measured by directEIA (DRG-instruments Germany).

Statistics: Any data were tested for normal Gauss distribution by KS-test. In the case of normal distribution MANOVA was carried out for the factors „time of measurement" or „drink" or „interaction". If significant with $p<0,05$ a T-test was used for further evaluation. If we found no normal distribution we used Friedmann's test and post hoc Wilcoxon's test.

Results and Interpretation

The behaviour of the parameters hemoglobine, erythrocytes and hematocrite (tables 4,6,7) show, that by the heavy bicycle exercise a hemoconcentration took place and in the recovery phase when lying in the supine position a fluid redistribution from the interstitial space occured. Therefore all other blood parameters had to be corrected for the plasma volume shift according to the formula of Dill and Costill which involves the differences in hemoglobine and hematocrite values. In the present study we used measurement M5 in the recovered state as reference=100% and corrected the values of the measurements M1 – M4. In the tables, except the above mentioned, all blood measurements are given as corrected values. Any results were documented in means and standard deviations since most of the parameters were in a normal Gauss distribution. Table 21 shows the parameters being not normally distributed.

Circulatory and blood parameters

The mean values of work load (336 Watt, table 1) and heart frequency (186, table 3) underline the heavy exercise. The severity of the resulting stress reaction can be shown by the increased values of leukocytes (table 5), blood glucose (table 9), catecholamines (tables 13,14,15), and serotonine (table 16) directly after exercise (M1), and by the elevated concentrations of prolactine and cortisole (tables 11 and 12) at the measurements 44 and 59 min after exercise stress (M2 and M3). These physiological reactions are in the range somewhat below values that are reached from athletes in competitions.

Within 2 hours of recovery all blood parameters returned to normal values i.e. to the circadianic rhythm. Curves of mean values are shown for prolactine in fig. 12, for epinephrine and norepinephrine in fig. 13, and for dopamine and serotonine in fig. 14. MANOVA (table 22) or Friedmann's test (table 23) revealed significant changes along with recovery time for any blood parameter. Between the drinks significant differences were only found for hematocrite and prolactine (fig.12). So L-theanine does not influence the behaviour of hormones being important for adaptation or regeneration i.e. stabilisation of energy metabolism.

By post hoc tests for prolactine we could not find a distinct time being responsible for the sign. differences between the drinks. But the curve of drink 3 (200mg of L-theanine) is lower at any time. So a shift effect of L-theanine on the curve can be assumed in that the reset to normal values during recovery is accelerated. Correlations with cortisol (tables 27,28,29) let suppose a general

pituitary reaction which must be discussed in connection with the fact that correlations of prolactine with power in several EEG frequency bands occur only after theanine containing drinks. Other correlations between EEG-parameters and hormones are altered by the drinks too (see below, tables 27,28,29). The most important link between central nervous regulation and the peripheral operators of regulation (hormones) is the hypothalamic-pituitary system where neurotransmitters regulate peripheral reactions like that of prolactine via dopamine (inhibiting) and serotonine (activating) at the hypothalamic level. From animal studies it is known that theanine can influence both of these transmitter systems. In the present study this can be assumed indirectly only. But the physiological regulating roles seem not to be disturbed or inhibited.

Heart frequency and blood pressure values (table 3) returned very fast to normal resting values without differences between the test trials.

EEG measurements

In table 19 and figures 8, 9 and 10 EEG power values of 17 electrodes are averaged. The mean values in all frequency bands show a high electric activity of the brain cortex directly after severe exercise. This is in accordance with previous findings. Theta power statistically (with high variations) remains at an elevated level up to M 4 and than declines sign.. In all other frequeny bands even at M2 power values are reduced and then remain nearly at the same level or show a slight increase from M4 (74 min after end of exercise) to M5 (104 min after exercise). By statistical analyses no effect of the drinks could be shown in any frequency, only effects of time after exercise.

Figure 7 shows the glow mode qualitative analyses of 3 frequency bands with placebo as the example for the firstly demonstrated recovery course after strenious exercise. In this mode low frequencies (delta) are given in red colour, high frequencies in green (alpha) and blue (beta) and the power is represented with dark (low power) to bright (high power) in the respective colour. This figure shows in the most single electrode positions (left) and the map (right) a clear dominance of high frequency bands (yellow and green) and a high activity (brightness) directly after exercise (M1) especially in the central regions. During the recovery phase there is a shift to lower frequencies (red) especially in the frontal areas at M3 and a decrease in power (dark) in central areas at M2, temporal areas at M3 and M4. Later than 90 min after exercise (compare M4 with M5) there is a kind of rebound to higher activity levels and frequency bands (shift to green and yellow). The reason might be that more than two hours after exercise the probands get hungry. No qualitative or semiquantitaive differences between the drinks could be seen with this methodes.

With the methode of statistic maps like shown in figure 11 a particular influence of L-theanine on the electrical brain activity in single electrode positions is demonstrable. When compared placebo with 200 mg of L-theanine (drink 1 vs 3), the power at M2 as percentage of the power at M1 showed trends (small

squares) and sign. (big squares) differences in selected electrode positions in the bands of alpha 2, beta 1 and 2. As figure 11 demonstrates, the reduction of activity is accelerated with L-theanine in the early recovery phase with $p<0.1$ in alpha2 in the frontal, occipital and right temporal region, and with $p<0.05$ in beta1 and 2 in single parietal and occipital positions. The same trend can be seen for delta at M1 left central whereas delta showed at M4 a somewhat higher activity in a single left central and occipital electrode (C3).

Electrosympathography (table 20)

Low values of skin resistance [kOhm] directly after exercise (nb) are thought to be the result of high sympathetic activity. Changes in the time course are highly sign. but don´t reach the initial preexercise (vb) levels within 2 hours. Differences between the drinks could not be found.

CyclicAMP

Fig. 13 and table 30 demonstrate the well known up-regulation of the receptor sensitivity (stimulation with Isoproterenol) and increased basal cell activity (not stimulated) directly after severe exercise with high sympathetic activation. Up to M4 a down-regulation took place and in the last hour of the experiment a slight increase could be shown. The behaviour in basal and stimulated values was strongly parallel and statistics showed no differences between stimulation with 10 or 100 mikroMol of Isoproterenol. So correlations were calculated only with the values from 10 mikroMol stimulation (see below). Statistical analysis (tables 31 – 33) revealed sign. differences beween measurement times (except M4 – M5 10mikroMol stimulated) but not between drinks. So theanine seemed not to influence receptor-adaptation or –regulation.

Urine samples and renal function

The excretion of the catecholamine and serotonine metabolites homovanillic acid and 5-hydroxy-indol-acetic acid was not different between trials when expressed as total mass nor when related to urine creatinine (tables 17 and 18). Urine volume produced within the collecting period and creatinine excretion was nearly the same in all trials. Although serum creatinine concentrations showed small n.s. differences between the drinks the creatinine clearance was not different.

Correlations

Tables 27,28 and 29 show correlations between peripheral hormones and electrical brain activity in different frequency bands and between hormones among each other. But correlations were not the same after all drinks. Those of dopamine with power in lower frequencies in the placebo trial disappeared in trials with L-theanine containing drinks, and instead of this new negative correlations of prolactine with alpha1 and beta1 power appeared. Furthermore correlations of theta power with dopamine and norepinephrine disappeared. The

same was true for small correlations of serotonine and cortisole with delta and beta1 i.e. alpha1 and beta1. Only the correlations of beta2 power with all 3 catecholamines remained stable throughout the trials.
Correlations with cAMP can be seen in table 34. As expected cAMP was correlated with norepinephrine throughout all trials, but with epinephrine only after drink 2, and with dopamine only after drink 3. The mean power of 17 electrodes in delta frequency was corrrelated with cAMP in all trials. Theta and alpha1 power correlations with cAMP in the trial with placebo disappeared in the trials with low (drink 2) or high (drink3) L-theanine concentrations.
The single electrode positions and frequency bands showing differences between drink 1 and 3 were Pz beta1 and O1 beta2 (see fig. 11). For those we calculated correlations too (see table 35). Thereby some other corelations could be found but no new aspects occured. The disappearence of correlations of EEG parameters with serotonine after ingestion of L-theanine was verified as well as the close connection between plasma-norepinephrine, partially -dopamin and -epinephrine, and beta-activity.

Discussion

The present study demonstrated how the recovery from severe exercise looks like in the electrical brain activity and in the behaviour of some circulating hormones. Correlations between those parameters could be shown too. The application of drinks containing L-theanine in a low and high dose did not influence the regulating function of hormones or the excretion of their metabolites nor did it influence the brain activity in a serious manner. The renal function was not affected. The MANOVA-tests established differences in the hematocrit values. Although not significant in post hoc tests at distinct measuring times this let suppose that the fluid exchange between the extra- and intravasal space is influenced. With respect to this phenomenon further research is needed.
In connection with reset mechanisms from stress other distinct effects are obviously demonstrable:

1) The statistic factor analysis showed influences on the serum prolactine levels in total but not on the time course or the time dependant reaction to preceding stress events. Since the prolactine secretion is regulated by dopamine and serotonine at the hypothalamic level, and since effects of L-theanine on this neurotransmitter system are known from animal experiments, it is unlike to suppose a comparable effect. Yet the global central reactions during regeneration are uninfluenced by the different drinks as shown by averaged EEG measurements. So probably the overall regulating functions of the brain are unaffected. But a distinct effectiveness of the L-theanine containing drinks is demonstrable by the statistic maps (fig.11) in the electrode positions parietal central in the beta1 frequency band and occipital 1 in beta2 frequency in the early recovery phase (M1) where with L-theanine the activities are faster reduced. Since these cortex areas belong to the sensitive

assoziation and processing regions, and since power in high frequency (beta) is related to excitation, one can interpretate this as an accelerated deactivation in the sensory integration processes because of better coping after stress. The somewhat higher activity in the delta frequency band in single electrodes in a later phase (M4, see fig. 11) point in the same direction. Since activities in the slow waves are related to mental conditions like sleep, diziness and relaxation, and activities in the fast waves to awake or even excitation, it is unlike to interprete these observations as a supporting effect on relaxation and mental recovery after stress without influencing the normal regeneration reactions.

2) The brain dopamine and serotonine systems are responsible for the up- and down-regulation of mental activity, for vigilance and motivation. The peripheral hormone prolactine is regulated by these systems. Prolactine reacts like a stress hormone with a delay into the regeneration phase. The very distinct effect of the L-theanine containing drinks on the prolactine levels and the changes of correlations between electrical brain activity and peripheral stress hormone systems let speculate, that coupling systems between the central nervous system and the peripheral endocrine regulation are influenced without changing the peripheral hormone levels and thereby without disturbing the regulating effectivity of stress hormones. From literature it is known, that L-theanine in high doses inhibits cAMP-mediated post-receptor reactions. In the current study statistical analysis showed no differences between the trials in the basal and stimulated cAMP production in vivo of lymphocytes isolated directly after stress or in the regeneration phase. So we could not find an influence of L-theanine on the reset of upregulated postreceptor sensitivity and activity.

3) As a result the altered correlations between electrical cortex activity and hormonal parameters i.e. the coupling between brain and peripheral stress systems are not influenced at the level of beta-adrenergic receptors. But with our study design we only can estimate the phase of downregulation from elevated receptor sensitivity. Possibly the upregulation i.e. the behaviour of stimulated cAMP production may be influenced when L-theanine was applicated before stress. Another problem with interpretation of cellular reactions after exercise is the change in cell populations and subsets with different receptor densities. Perhaps studies in cultered cells while incubation with L-theanine will lead to other results.

The interindividual alpha wave activity has a very high variability. People can divided into high and low alpha types. With respect to the alpha types we made subgroupes with high and low alpha activity and repeated the statistcal analyses, but found no differences between neither groups.

Conclusions

The single regular hormonal functions and the normal general cortical activities are uninfluenced by drinks containing 50 or 200 mg L-theanine. So normal

functions are not restricted. But differences between placebo and verum trials were found in the behaviour of serum prolactine, which is dopamine and serotonine regulated and reacts like a stress hormone. This as well as the altered correlations between EEG-parameters and peripheral hormone concentrations show, that L-theanine influences the coupling between central and peripheral regulation. This seems not to be mediated by influencing the beta-receptor or postreceptor regulation.

The enhanced reset of beta activity in sensory regions in the early recovery phase after physical stress is in accordance with conclusions in the literature about the relaxing effect. In the present study severe physical exercise was used for stimulation. In several published studies in animal models caffeine served as stimulant. Although these models are very different each demonstrated a downregulating effect on the current stimulus. Another recent study found the generation of alpha waves after ingestion of L-theanine. This is known to be correlated with a relaxed awaken state. Alltogether there is evidence enough for the statement that L-theanine induces relaxing mechanisms independant of dosage by support of physiological reactions and not by a pharmacological effect.

<u>Tables</u>

1. Anthropometric and ergometer data of test persons
2. List of randomization
3. Heart frequency and blood pressure
4. Hemoglobine
5. Leucocytes
6. Erythrocytes
7. Hematocrite
8. Thrombocytes
9. Blood glucose
10. Serum creatinine
11. Prolactine
12. Cortisole
13. Dopamine
14. Epinephrine
15. Norepinephrine
16. Serotonine
17. Urine values /concentrations
18. Urine values in absolute mass (mg) and calculated for mg creatinine
19. EEG data as average of 17 electrode positions
20. Electrosympathography data
21. Kolmogoroff-Smirnov-test for normal distribution
22. MANOVA results
23. ANOVA results for urine parameters
24. Friedmann-test
25. T-Test
26. Wilcoxon-Test
27. Correlations drink 1
28. Correlations drink 2
29. Correlations drink 3
30. CyclicAMP
31. Kolmogoroff-Smirnov-test for normal distribution for cAMP
32. MANOVA and Friedmann-test cAMP
33. T-test and Wilcoxon-test cAMP
34. Correlations cAMP
35. Correlations selected beta1 Pz- and beta2 O1-electrodes

Table 1:
Anthropometric and ergometer data

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
age	14	25	2	14	25	2	14	25	2
height	14	181,2	4,9	14	181,2	4,9	14	181,2	4,9
weight	14	78,8	8,7	14	79,0	9,4	14	79,1	9,7
max. watt	14	336	36	14	336	36	14	338	36
wattsteps	14	67	7	14	67	7	14	67	7

Table 2:
List of randomization

		Week								
		1			2			3		
		drink			drink			drink		
		1	2	3	1	2	3	1	2	3
Probandnumber	1		x		x					x
	2			x		x		x		
	3	x				x				x
	4			x		x		x		
	5		x		x					x
	6			x	x				x	
	7	x					x		x	
	8		x		x					x
	9	x					x		x	
	10	x					x		x	
	12	x					x		x	
	13		x		x					x
	14	x				x				x
	15		x		x					x

Table 3:
Heart frequency [s/min] and blood pressure s(ystolic) and d(diastolic) [mmHg]

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
hfmax	14	186	10	14	185	7	14	184	9
hf2min	14	113	18	14	115	14	14	115	18
hf60min	14	71	8	14	70	8	14	70	9
hf120min	14	65	10	14	65	8	14	63	10
hf60_120	14	67	8	14	66	7	14	67	9
bps_1	14	123	13	14	122	12	14	127	14
bps_2	14	114	12	14	116	11	14	117	7
bps_3	14	114	11	14	113	11	14	114	5
bps_4	14	112	8	14	111	13	14	111	6
bps_5	14	117	11	14	112	12	14	111	7
bpd_1	14	63	12	14	63	11	14	64	14
bpd_2	14	71	8	14	69	8	14	73	6
bpd_3	14	71	9	14	68	8	14	73	6
bpd_4	14	71	6	14	67	6	14	70	6
bpd_5	14	75	7	14	75	9	14	75	5

Table 4:
Hemoglobine [g/100ml]

	drink 1			drink 2			drink 3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
hb_m1	14	16,07	1,11	14	15,96	1,16	14	15,85	,93
hb_m2	14	14,00	1,01	14	13,78	,95	14	13,76	,84
hb_m3	14	13,79	1,02	14	13,70	,97	14	13,78	,76
hb_m4	14	14,07	1,11	14	13,86	1,05	14	13,84	,79
hb_m5	14	14,16	,94	14	14,43	1,42	14	14,00	,78

Table 5:
Leucocytes [10^3/µl]

	drink 1			drink 2			drink 3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
leuc_m1	14	7,24	1,73	14	7,16	1,72	14	7,55	2,38
leuc_m2	14	4,45	1,19	14	4,04	2,03	14	4,91	1,85
leuc_m3	14	4,55	1,29	14	4,55	1,38	14	4,98	1,87
leuc_m4	14	4,71	1,21	14	4,86	1,47	14	5,27	1,93
leuc_m5	14	5,51	1,47	14	5,27	1,39	14	5,79	1,99

Table 6:
Erythrocytes [10^7/µl]

	drink 1			drink 2			drink 3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
ery_m1	14	5,35	,38	14	5,35	,43	14	5,29	,37
ery_m2	14	4,69	,32	14	4,59	,34	14	4,65	,32
ery_m3	14	4,61	,32	14	4,57	,32	14	4,57	,30
ery_m4	14	4,65	,33	14	4,61	,38	14	4,58	,28
ery_m5	14	4,74	,30	14	4,69	,32	14	4,68	,31

Table 7:
Hematocrite [L/L]

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
hct_m1	14	,45	,03	14	,45	,03	14	,44	,02
hct_m2	14	,39	,03	14	,38	,02	14	,39	,03
hct_m3	14	,38	,02	14	,38	,02	14	,38	,02
hct_m4	14	,39	,02	14	,38	,03	14	,38	,02
hct_m5	14	,40	,03	14	,39	,02	14	,39	,02

Table 8:
Thrombocytes [10^6/µl] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
thro_m1k	14	256,05	61,96	14	278,33	104,12	14	266,29	70,97
thro_m2k	14	241,23	66,70	14	251,17	90,87	14	249,77	72,95
thro_m3k	14	251,87	77,11	14	246,91	91,84	14	242,60	78,62
thro_m4k	14	251,36	72,89	14	260,01	100,45	14	246,52	81,25
thro_m5k	14	249,14	82,82	14	246,00	74,03	14	261,00	82,66

Table 9:
Blood Glucose [mmol/l] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
gluc_m1k	14	4,27	,78	14	4,12	,73	14	4,08	,57
gluc_m2k	14	7,36	1,26	14	7,59	,90	14	7,29	1,13
gluc_m3k	14	6,72	,60	14	6,49	,81	14	6,35	,94
gluc_m4k	14	5,45	,77	14	5,21	,88	14	5,07	,66
gluc_m5k	14	5,17	,63	14	5,13	,45	14	5,13	,71

Table 10:
Serum Creatinine [mg/100ml] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw.	Sd
kr_s_m1k	14	,89	,12	14	,88	,08	14	,89	,08
kr_s_m2k	14	1,02	,13	14	1,03	,08	14	1,02	,11
kr_s_m3k	14	1,05	,10	14	1,03	,10	14	1,01	,09
kr_s_m4k	14	1,01	,11	14	1,01	,09	14	1,01	,10
kr_s_m5k	14	,99	,09	14	,97	,07	14	,99	,08

Table 11:
Serum Prolactine [mIU/l] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
pr_s_m1k	14	196,92	75,38	14	206,73	80,28	14	192,24	85,45
pr_s_m2k	14	228,80	97,17	14	239,73	120,37	14	218,77	111,19
pr_s_m3k	14	223,38	107,10	14	223,81	110,31	14	212,01	99,61
pr_s_m4k	14	201,46	101,89	14	206,22	97,45	14	194,99	100,94
pr_s_m5k	14	175,36	100,80	14	170,93	91,05	14	158,36	87,28

Table 12:
Serum Cortisole [µg/dl] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
co_s_m1k	14	11,45	2,58	14	12,46	3,92	14	11,80	3,25
co_s_m2k	14	13,71	2,60	14	12,67	3,49	14	11,94	2,44
co_s_m3k	14	12,45	2,94	14	11,72	3,45	14	10,37	2,16
co_s_m4k	14	11,01	2,86	14	10,43	3,02	14	9,80	2,28
co_s_m5k	14	9,39	3,42	14	8,44	2,30	14	8,60	3,09

Table 13:
Plasma Dopamine [nmol/l] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
do_s_1kk	14	,49	,21	14	,60	,26	14	,50	,22
do_s_2kk	14	,27	,11	14	,32	,18	14	,29	,13
do_s_3kk	14	,26	,11	14	,34	,16	14	,32	,14
do_s_4kk	14	,33	,11	14	,30	,14	14	,32	,12
do_s_5kk	14	,33	,13	14	,29	,16	14	,32	,13

Table 14:
Plasma Epinephrine [nmol/l] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
ad_s_1kk	14	2,18	2,57	14	1,50	1,10	14	1,71	2,19
ad_s_2kk	14	,21	,15	14	,26	,18	14	,32	,27
ad_s_3kk	14	,21	,14	14	,25	,16	14	,24	,13
ad_s_4kk	14	,21	,10	14	,29	,17	14	,24	,07
ad_s_5kk	14	,25	,13	14	,25	,12	14	,37	,27

Table 15:
Plasma Norepinephrine [nmol/l] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
no_s_1kk	14	12,41	5,50	14	10,95	4,94	14	10,45	5,08
no_s_2kk	14	,99	,47	14	1,02	,56	14	,87	,45
no_s_3kk	14	,89	,62	14	1,00	,74	14	,87	,45
no_s_4kk	14	1,07	,45	14	,84	,38	14	1,09	,48
no_s_5kk	14	1,23	,54	14	1,10	,41	14	1,13	,48

Table 16:
Serum Serotonine [µg/l] (corrected for plasma volume shifts with m5 = 100%)

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
ser_m1k	14	158,18	95,95	14	133,52	68,20	14	128,57	94,57
ser_m2k	14	40,86	39,79	14	42,06	22,09	14	62,14	61,58
ser_m3k	14	42,83	34,94	14	43,54	26,40	14	40,95	38,26
ser_m4k	14	37,83	30,02	14	44,03	50,10	14	33,25	24,77
ser_m5k	14	43,79	40,56	14	55,43	64,42	14	61,14	43,13

Table 17:
Urine values [mg/l]

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
Kreatinin im Urin	14	,68	,48	14	,71	,57	14	,59	,41
5-HIES im Urin	14	1,83	1,21	14	2,14	1,77	14	1,69	,94
Homovanillinsäure im Urin	14	3,12	2,16	14	3,49	3,14	14	2,36	1,50

Table 18:
Urine values as absolute values [mg] and calculated for urine-creatin [mg/mg creatinine]

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
Creatinine	14	,18	,03	14	,18	,02	14	,18	,02
5-HIES	14	,50	,09	14	,60	,45	14	,59	,33
Homovanillinsäure	14	,88	,32	14	,89	,51	14	,78	,35
Homovanillin/ Creatinine	14	16,3	9,52	14	18,9	16,2	14	13,0	8,01
5 HIES/Creatinine	14	9,50	4,65	14	11,6	9,15	14	9,40	5,34

Table19:
EEG data (average of 17 electrode positions)
d= delta power[μV^2], t= theta power[μV^2]
a1= alpha1 power[μV^2], a2= alpha2 power[μV^2]
b1= beta1 power[μV^2], b2= beta2 power[μV^2]

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
d_mw_m1	14	27,51	29,91	14	21,19	14,18	14	18,95	11,66
d_mw_m2	14	12,11	8,32	14	15,95	8,54	14	11,25	6,86
d_mw_m3	14	12,29	9,28	14	15,94	11,63	14	11,77	7,39
d_mw_m4	14	10,36	8,12	14	11,80	7,55	14	13,41	9,08
d_mw_m5	14	9,06	3,89	14	9,66	5,23	14	8,51	4,32
t_mw_m1	14	3,65	3,20	14	3,99	4,02	14	3,21	2,40
t_mw_m2	14	2,57	1,57	14	4,00	3,27	14	2,84	2,11
t_mw_m3	14	3,24	2,29	14	3,83	2,94	14	3,11	2,02
t_mw_m4	14	2,59	1,52	14	3,37	2,96	14	3,41	2,19
t_mw_m5	14	2,72	1,99	14	2,94	3,49	14	2,58	2,00
a1_mw_m1	14	8,91	12,20	14	9,94	14,09	14	8,70	11,18
a1_mw_m2	14	4,43	3,17	14	8,75	16,70	14	7,65	11,79
a1_mw_m3	14	7,38	13,08	14	8,22	12,82	14	7,72	11,12
a1_mw_m4	14	5,11	4,86	14	8,92	13,49	14	6,04	9,21
a1_mw_m5	14	8,55	11,29	14	9,98	14,44	14	9,46	12,02
a2_mw_m1	14	15,89	11,13	14	17,02	13,64	14	17,38	14,70
a2_mw_m2	14	12,86	12,86	14	8,47	12,03	14	12,11	14,05
a2_mw_m3	14	9,58	14,28	14	8,68	11,77	14	10,51	11,91
a2_mw_m4	14	10,96	13,52	14	10,51	13,90	14	10,50	16,54
a2_mw_m5	14	12,96	14,81	14	12,71	16,20	14	12,58	15,12
b1_mw_m1	14	2,78	1,43	14	2,72	1,36	14	2,68	1,35
b1_mw_m2	14	1,87	,76	14	1,97	,97	14	1,83	,92
b1_mw_m3	14	2,03	,92	14	2,24	1,38	14	1,90	,86
b1_mw_m4	14	1,61	,76	14	1,81	,94	14	1,86	,97
b1_mw_m5	14	1,81	,84	14	1,91	,89	14	1,84	,86
b2_mw_m1	14	2,14	,93	14	2,16	1,07	14	2,16	1,14
b2_mw_m2	14	1,57	,74	14	1,41	,71	14	1,55	,86
b2_mw_m3	14	1,39	,73	14	1,49	,71	14	1,41	,85
b2_mw_m4	14	1,44	,76	14	1,43	,78	14	1,33	,93
b2_mw_m5	14	1,56	,75	14	1,63	,87	14	1,54	,85

Table 20:
Elektrosympathograhy [Ohm] vb=before exercise, nb=vb=before exercise, 15, .. min. after exercise

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
esgvb	14	453.8	86.4	14	444.5	70.6	14	450.7	81.0
esgnb	14	389.2	15.3	14	390.2	23.0	14	388.7	11.3
esg_15	14	376.4	14.2	14	374.9	19.0	14	375.3	9.0
esg_30	14	395.9	39.2	14	413.2	35.1	14	379.5	73.2
esg_45	14	407.7	44.9	14	411.4	34.0	14	403.9	30.1
esg_60	14	417.2	49.3	14	416.1	35.7	14	414.4	37.6
esg_75	14	418.1	58.6	14	414.1	38.7	14	418.0	43.9
esg_135	14	414.0	52.7	14	413.8	44.8	14	413.6	45.1

Statistic L-Theanin :

Table 21:

KS TEST:

All normal Gaussian variable, except for:

parameter	
blood pressure systolic m1	p=0,023*
blood pressure systolic m2	p=0,031*
blood pressure systolic m3	p=0,047*
blood pressure diastolic m1	p=0,033*
blood pressure diastolic m2	p=0,016*
blood pressure diastolic m3	p=0,010*
blood pressure diastolic m4	p=0,004**
blood pressure diastolic m5	p=0,002**
Hematocrite m3	p=0,047*
eeg theta m2	p=0,029*
eeg theta m5	p=0,021*
eeg alpha1 m1	p=0,002**
eeg alpha1 m2	p=0,001**
eeg alpha1 m3	p=0,000**
eeg alpha1 m4	p=0,001**
eeg alpha1 m5	p=0,017*
eeg alpha2 m1	p=0,013*
eeg alpha2 m2	p=0,009**
eeg alpha2 m3	p=0,003**
eeg alpha2 m4	p=0,004**
eeg alpha2 m5	p=0,006**
serum prolactine m1	p=0,042*
serum prolactine m2	p=0,006**
serum prolactine m3	p=0,025*
serum prolactine m4	p=0,006**
plasma epinephrine m1	p=0,017*
plasma epinephrine m2	p=0,024*
plasma epinephrine m5	p=0,022*
5-HIES absolute	p=0,002**
homovanillineacid absolute	p=0,017*
creatine concentration	p=0,034*

Table 22:

MANOVA:

parameter	(M1-M2-M3-M4-M5)	(drink 1-2-3)
heart frequency	p=0,000**	p=0,817
leucocytes	p=0,000**	p=0,666
erythrocytes	p=0,000**	p=0,883
hemoglobine	p=0,000**	p=0,881
thrombocytes	p=0,000**	p=0,976
blood glucose	p=0,000**	p=0,471
serum creatinin	p=0,000**	p=0,960
serum cortisole	p=0,000**	p=0,498
plasma dopamine	p=0,000**	p=0,669
plasma norephinephrine	p=0,000**	p=0,644
serum serotonine	p=0,000**	p=0,992
esg	p=0,000**	p=0,922
eeg delta	p=0,000**	p=0,748
eeg beta1	p=0,000**	p=0,886
eeg beta2	p=0,000**	p=0,998

Table 23:

ANOVA for the urine parameters:

parameter	(drink 1-2-3)
creatine absolute	p=0,933
5-HIES concentration	p=0,669
homovanillineacid concentration	p=0,444
homovanillineacid/creatine	p=0,425
5 hies/creatine	p=0,621

Table 24:

FRIEDMANN TEST:

parameter	(M1-M2-M3-M4-M5)
blood pressure systolic	p=0,000**
blood pressure diastolic	p=0,000**
hematocrite	p=0,000**
eeg theta	p=0,030*
eeg alpha1	p=0,000**
eeg alpha2	p=0,000**
serum prolactine	p=0,000**
plasma epinephrine	p=0,000**

parameter	(drink 1-2-3)
blood pressure systolic	p=0,713
blood pressure diastolic	p=0,079
hematocrite	p=0,002**
eeg theta	p=0,324
eeg alpha1	p=0,407
eeg alpha2	p=0,296
serum prolactine	p=0,009**
plasma epinephrine	p=0,173
5-HIES absolute	p=0,424
homovanillineacid absolute	p=0,395
creatine concentration	p=0,335

parameter	(drink 1-2-3)
hematocrite m1	p=0,284
hematocrite m2	p=0,122
hematocrite m3	p=0,521
hematocrite m4	p=0,076
hematocrite m5	p=0,254
serum prolactine m1	p=0.931
serum prolactine m2	p=0,135
serum prolactine m3	p=0,168
serum prolactine m4	p=0,145
serum prolactine m5	p=0,607

Table 25a:

T- TEST:

parameter	measurement	
heart frequency	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
leucocytes	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
erythrocytes	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
hemoglobine	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
thrombocytes	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,021*
	m1-m5	p=0,045*
blood glucose	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
serum creatinae	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
serum cortisole	m1-m2	p=0,092
	m1-m3	p=0,418
	m1-m4	p=0,002**
	m1-m5	p=0,000**
plasma dopamine	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
plasma norephinephrine	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
serum serotonine	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
ese	m1-15min	p=0,000**
	m1-30min	p=0,370
	m1-45min	p=0,001**
	m1-60min	p=0,000**
	m1-75min	p=0,001**
	m1-135min	p=0,002**

Table 25b:

parameter	measurement	
eeg delta	m1-m2	p=0,001**
	m1-m3	p=0,004**
	m1-m4	p=0,001**
	m1-m5	p=0,000**
eeg beta1	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**
eeg beta2	m1-m2	p=0,000**
	m1-m3	p=0,000**
	m1-m4	p=0,000**
	m1-m5	p=0,000**

Table 26:

WILCOXON TEST:

parameter	measurement	
blood pressure systolic	m1-m2	p=0,000**
blood pressure systolic	m1-m3	p=0,000**
blood pressure systolic	m1-m4	p=0,000**
blood pressure systolic	m1-m5	p=0,000**
blood pressure diastolic	m1-m2	p=0,001**
blood pressure diastolic	m1-m3	p=0,001**
blood pressure diastolic	m1-m4	p=0,001**
blood pressure diastolic	m1-m5	p=0,000**
hematocrite	m1-m2	p=0,000**
hematocrite	m1-m3	p=0,000**
hematocrite	m1-m4	p=0,000**
hematocrite	m1-m5	p=0,000**
eeg theta	m1-m2	p=0,297
eeg theta	m1-m3	p=0,803
eeg theta	m1-m4	p=0,328
eeg theta	m1-m5	p=0,000**
eeg alpha1	m1-m2	p=0,008**
eeg alpha1	m1-m3	p=0,006**
eeg alpha1	m1-m4	p=0,003**
eeg alpha1	m1-m5	p=0,740
eeg alpha2	m1-m2	p=0,000**
eeg alpha2	m1-m3	p=0,000**
eeg alpha2	m1-m4	p=0,000**
eeg alpha2	m1-m5	p=0,000**
serum prolactine	m1-m2	p=0,000**
serum prolactine	m1-m3	p=0,002**
serum prolactine	m1-m4	p=0,945
serum prolactine	m1-m5	p=0,000**
plasma epinephrine	m1-m2	p=0,000**
plasma epinephrine	m1-m3	p=0,000**
plasma epinephrine	m1-m4	p=0,000**
plasma epinephrine	m1-m5	p=0,000**

parameter	drink	
hematocrite	d1-d2	p=0,062
hematocrite	d1-d3	p=0,010*
hematocrite	d2-d3	p=0,326
serum prolactine	d1-d2	p=0,911
serum prolactine	d1-d3	p=0,027*
serum prolactine	d2-d3	p=0,001**

		delta	teta	alpha1	alpha2	beta1	beta2	dopamin	adrenalin	noradrenalin	serotonin	cortisol	prolaktin
	Korrelation nach Pearson		,747	,486		,518	,264	,340	,270	,421	,310		
	Signifikanz (2-seitig)		,000	,000		,000	,027	,004	,024	,000	,009		
	N		70	70		70	70	70	70	70	70		
	Korrelation nach Pearson	,747		,709		,404		,315		,247			
	Signifikanz (2-seitig)	,000		,000		,001		,008		,039			
	N	70		70		70		70		70			
a1	Korrelation nach Pearson	,486	,709			,306		,239				-,238	
	Signifikanz (2-seitig)	,000	,000			,010		,047				,048	
	N	70	70			70		70				70	
a2	Korrelation nach Pearson					,530	,701						
	Signifikanz (2-seitig)					,000	,000						
	N					70	70						
1	Korrelation nach Pearson	,518	,404	,306	,530		,718			,412	,295	-,266	
	Signifikanz (2-seitig)	,000	,001	,010	,000		,000			,000	,013	,026	
	N	70	70	70	70		70			70	70	70	
2	Korrelation nach Pearson	,264			,701	,718		,245	,315	,408			
	Signifikanz (2-seitig)	,027			,000	,000		,041	,008	,000			
	N	70			70	70		70	70	70			
amin	Korrelation nach Pearson	,340	,315	,239			,245		,635	,653	,424		
	Signifikanz (2-seitig)	,004	,008	,047			,041		,000	,000	,000		
	N	70	70	70			70		70	70	70		
nalin	Korrelation nach Pearson	,270					,315	,635		,613	,335		
	Signifikanz (2-seitig)	,024					,008	,000		,000	,005		
	N	70					70	70		70	70		
drenalin	Korrelation nach Pearson	,421	,247			,412	,408	,653	,613		,667		
	Signifikanz (2-seitig)	,000	,039			,000	,000	,000	,000		,000		
	N	70	70			70	70	70	70		70		
tonin	Korrelation nach Pearson	,310				,295		,424	,335	,667			
	Signifikanz (2-seitig)	,009				,013		,000	,005	,000			
	N	70				70		70	70	70			
sol	Korrelation nach Pearson			-,238		-,266							,527
	Signifikanz (2-seitig)			,048		,026							,000
	N			70		70							70
aktin	Korrelation nach Pearson											,527	
	Signifikanz (2-seitig)											,000	
	N											70	

		delta	teta	alpha1	alpha2	beta1	beta2	dopamin	adrenalin	noradrenalin	serotonin	cortisol	prolaktin
a	Korrelation nach Pearson		,666	,389		,551	,363	,309	,385	,396			
	Signifikanz (2-seitig)		,000	,001		,000	,002	,010	,001	,001			
	N		69	69		69	69	69	69	69			
	Korrelation nach Pearson	,666		,859		,478							-,282
	Signifikanz (2-seitig)	,000		,000		,000							,019
	N	69		69		69							69
a1	Korrelation nach Pearson	,389	,859			,394						-,336	-,390
	Signifikanz (2-seitig)	,001	,000			,001						,005	,001
	N	69	69			69						69	69
a2	Korrelation nach Pearson					,508	,749	,345		,333			
	Signifikanz (2-seitig)					,000	,000	,004		,005			
	N					69	69	69		69			
1	Korrelation nach Pearson	,551	,478	,394	,508		,609			,334		-,268	-,338
	Signifikanz (2-seitig)	,000	,000	,001	,000		,000			,005		,026	,005
	N	69	69	69	69		69			69		69	69
2	Korrelation nach Pearson	,363			,749	,609		,329	,367	,412			
	Signifikanz (2-seitig)	,002			,000	,000		,006	,002	,000			
	N	69			69	69		69	69	69			
amin	Korrelation nach Pearson	,309			,345		,329		,429	,563	,275		,287
	Signifikanz (2-seitig)	,010			,004		,006		,000	,000	,021		,016
	N	69			69		69		70	70	70		70
nalin	Korrelation nach Pearson	,385					,367	,429		,734	,555		
	Signifikanz (2-seitig)	,001					,002	,000		,000	,000		
	N	69					69	70		70	70		
drenalin	Korrelation nach Pearson	,396			,333	,334	,412	,563	,734		,564		
	Signifikanz (2-seitig)	,001			,005	,005	,000	,000	,000		,000		
	N	69			69	69	69	70	70		70		
tonin	Korrelation nach Pearson							,275	,555	,564		,239	
	Signifikanz (2-seitig)							,021	,000	,000		,046	
	N							70	70	70		70	
isol	Korrelation nach Pearson			-,336		-,268					,239		,323
	Signifikanz (2-seitig)			,005		,026					,046		,006
	N			69		69					70		70
aktin	Korrelation nach Pearson		-,282	-,390		-,338		,287				,323	
	Signifikanz (2-seitig)		,019	,001		,005		,016				,006	
	N		69	69		69		70				70	

		delta	teta	alpha1	alpha2	beta1	beta2	dopamin	adrenalin	noradrenalin	serotonin	cortisol	prolaktin
lta	Korrelation nach Pearson		,652			,494				,410			
	Signifikanz (2-seitig)		,000			,000				,000			
	N		70			70				70			
a	Korrelation nach Pearson	,652		,697		,360							
	Signifikanz (2-seitig)	,000		,000		,002							
	N	70		70		70							
ha1	Korrelation nach Pearson		,697			,284							-,293
	Signifikanz (2-seitig)		,000			,017							,014
	N		70			70							70
ha2	Korrelation nach Pearson					,644	,761						
	Signifikanz (2-seitig)					,000	,000						
	N					70	70						
ta1	Korrelation nach Pearson	,494	,360	,284	,644		,786			,366			-,244
	Signifikanz (2-seitig)	,000	,002	,017	,000		,000			,002			,042
	N	70	70	70	70		70			70			70
ta2	Korrelation nach Pearson				,761	,786		,357	,305	,369			
	Signifikanz (2-seitig)				,000	,000		,002	,010	,002			
	N				70	70		70	70	70			
pamin	Korrelation nach Pearson						,357		,492	,544	,390		
	Signifikanz (2-seitig)						,002		,000	,000	,001		
	N						70		70	70	70		
renalin	Korrelation nach Pearson						,305	,492		,629			
	Signifikanz (2-seitig)						,010	,000		,000			
	N						70	70		70			
radrenalin	Korrelation nach Pearson	,410				,366	,369	,544	,629		,350		
	Signifikanz (2-seitig)	,000				,002	,002	,000	,000		,003		
	N	70				70	70	70	70		70		
rotonin	Korrelation nach Pearson							,390		,350			
	Signifikanz (2-seitig)							,001		,003			
	N							70		70			
rtisol	Korrelation nach Pearson												,32
	Signifikanz (2-seitig)												,00
	N												7
olaktin	Korrelation nach Pearson			-,293		-,244						,329	
	Signifikanz (2-seitig)			,014		,042						,005	
	N			70		70						70	

Table 30:
CAMP

	drink								
	1			2			3		
	n	Mw	Sd	n	Mw	Sd	n	Mw	Sd
Camp not stimulated M1	14	25,94	8,77	14	24,43	10,75	14	28,43	15,84
Camp not stimulated M4	14	10,40	2,38	14	11,53	3,79	14	14,00	5,79
Camp not stimulated M5	14	13,18	5,58	14	14,11	5,61	14	13,86	8,06
Camp stimulated with 10µmol/l M1	14	104,48	81,82	14	112,12	45,39	14	113,57	74,62
Camp stimulated with 10µmol/l M4	14	31,63	11,20	14	36,20	9,35	14	35,82	14,78
Camp stimulated with 10µmol/l M5	14	46,15	39,94	14	34,71	12,24	14	39,42	22,66
Camp stimulated with 100µmol/l M1	14	89,12	32,42	14	99,74	37,79	14	116,95	78,29
Camp stimulated with 100µmol/l M4	14	29,71	13,31	14	34,34	11,36	14	33,62	20,06
Camp stimulated with 100µmol/l M5	14	41,62	21,84	14	43,49	26,40	14	46,05	33,00

Statistic L-Theanin CAMP :

Table 31:

KS TEST:

All normal Gaussian variable, except for:

parameter	
Camp stimulated with 10µmol/l M5	p=0,025*
Camp stimulated with 100µmol/l M5	p=0,049*

Table 32a:

MANOVA:

parameter	(M1-M4-M5)	(drink 1-2-3)
Camp not stimulated	p=0,000**	p=0,595

Table 32b:

FRIEDMANN TEST:

parameter	(M1-M4-M5)
Camp stimulated with 10µmol/l	p=0,000**
Camp stimulated with 100µmol/l	p=0,000**

parameter	(drink 1-2-3)
Camp stimulated with 10µmol/l	p=0,212
Camp stimulated with 100µmol/l	p=0,636

Table 33a:

T-TEST

Parameter	measurement	
Camp not stimulated	m1-m4	p=0,000**
Camp not stimulated	m1-m5	p=0,000**
Camp not stimulated	m4-m5	p=0,0093
Camp stimulated with 10µmol/l	m1-m4	p=0,000**
Camp stimulated with 100µmol/l	m1-m4	p=0,000**

Table 33b:

WILCOXON TEST:

parameter	measurement	
Camp stimulated with 10µmol/l	m1-m5	p=0,000**
Camp stimulated with 10µmol/l	m4-m5	p=0,202
Camp stimulated with 100µmol/l	m1-m5	p=0,000**
Camp stimulated with 100µmol/l	m4-m5	p=0,000**

Table: 34

		Correlations Drink 1		Correlation Drink 2		Correlation Drink 3	
		Camp not stimulated	Camp stimulated	Camp not stimulated	Camp stimulated	Camp not stimulated	Camp stimulated
Camp not stimulated	Korrelation nach Pearson		,708**		,785**		,703**
	Signifikanz (2-seitig)		,000		,000		,000
	N		42		42		42
Camp stimulated	Korrelation nach Pearson	,708**		,785**		,703**	
	Signifikanz (2-seitig)	,000		,000		,000	
	N	42		42		42	
delta	Korrelation nach Pearson	,413**	,498**	,534**	,316*	,349*	,334*
	Signifikanz (2-seitig)	,007	,001	,000	,044	,024	,030
	N	42	42	41	41	42	42
theta	Korrelation nach Pearson	,361*	,287				
	Signifikanz (2-seitig)	,019	,066				
	N	42	42				
alpha 1	Korrelation nach Pearson	,391*	,317*				
	Signifikanz (2-seitig)	,011	,041				
	N	42	42				
alpha 2	Korrelation nach Pearson			,320*			
	Signifikanz (2-seitig)			,041			
	N			41			
beta 1	Korrelation nach Pearson			,530**	,347*		
	Signifikanz (2-seitig)			,000	,026		
	N			41	41		
beta 2	Korrelation nach Pearson			,472**			
	Signifikanz (2-seitig)			,002			
	N			41			
dopamine	Korrelation nach Pearson						,330*
	Signifikanz (2-seitig)						,033
	N						42
epinephrine	Korrelation nach Pearson			,590**	,572**		
	Signifikanz (2-seitig)			,000	,000		
	N			42	42		
norepinephrine	Korrelation nach Pearson	,622**	,644**	,635**	,719**	,440**	,533**
	Signifikanz (2-seitig)	,000	,000	,000	,000	,004	,000
	N	41	41	42	42	42	42
serotonine	Korrelation nach Pearson	,513**	,527**	,380*	,411**		
	Signifikanz (2-seitig)	,001	,000	,013	,007		
	N	42	42	42	42		
cortisole	Korrelation nach Pearson						
	Signifikanz (2-seitig)						
	N						
prolactine	Korrelation nach Pearson						
	Signifikanz (2-seitig)						
	N						

Table: 35

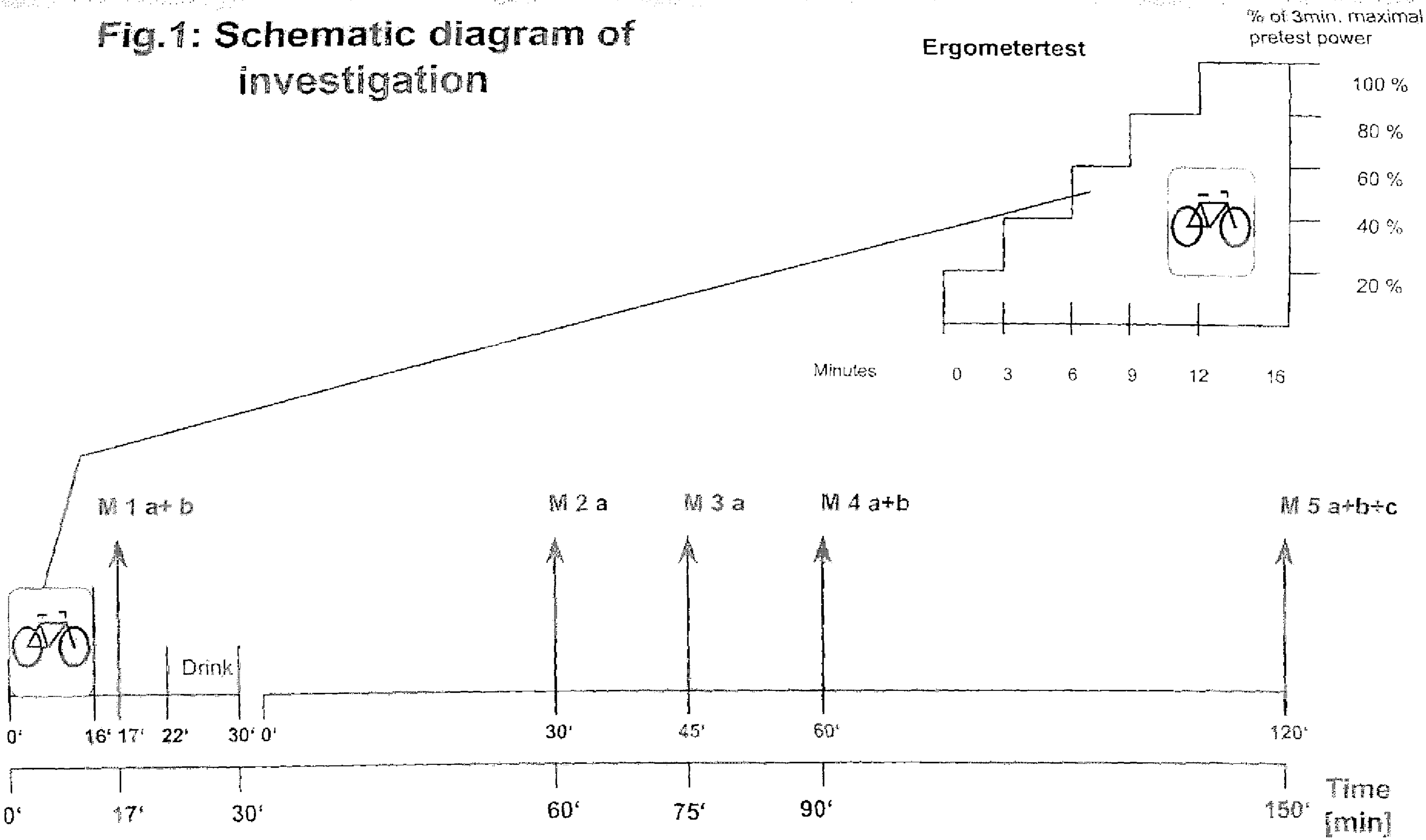
		Correlation drink1		Correlation drink2		Correlation drink3	
		beta1 pz	beta2 o1	beta1 pz	beta2 o1	beta1 pz	beta2 o1
ta1 pz	Korrelation nach Pearson		,513		,456		,679
	Signifikanz (2-seitig)		,000		,000		,000
	N		70		69		70
ia2 o1	Korrelation nach Pearson	,513		,456		,679	
	Signifikanz (2-seitig)	,000		,000		,000	
	N	70		69		70	
olactine	Korrelation nach Pearson	-,302		-,250			-
	Signifikanz (2-seitig)	,011		,038			-
	N	70		69			-
rtisole	Korrelation nach Pearson	-,361			-		-
	Signifikanz (2-seitig)	,002			-		-
	N	70			-		-
pamine	Korrelation nach Pearson		,287		,329		,434
	Signifikanz (2-seitig)		,016		,006		,000
	N		70		69		70
inephrine	Korrelation nach Pearson		,348	,255	,422	,252	,304
	Signifikanz (2-seitig)		,003	,034	,000	,036	,011
	N		70	69	69	70	70
repinephrine	Korrelation nach Pearson	,407	,497	,395	,512	,401	,527
	Signifikanz (2-seitig)	,000	,000	,001	,000	,001	,000
	N	70	70	69	69	70	70
otonine	Korrelation nach Pearson	,321	,349		-		-
	Signifikanz (2-seitig)	,007	,003		-		-
	N	70	70		-		-

		Correlation drink1		Correlation drink2		Correlation drink3	
		beta1 pz	beta2 o1	beta1 pz	beta2 o1	beta1 pz	beta2 o1
a1 pz	Korrelation nach Pearson		,589		,527		,704
	Signifikanz (2-seitig)		,000		,000		,000
	N		42		41		42
a2 o1	Korrelation nach Pearson	,589		,527		,704	
	Signifikanz (2-seitig)	,000		,000		,000	
	N	42		41		42	
p not ulated	Korrelation nach Pearson	,335		,574	,529		-
	Signifikanz (2-seitig)	,030		,000	,000		-
	N	42		41	41		-
p stimulated 10µmol/l	Korrelation nach Pearson		-		,384		-
	Signifikanz (2-seitig)		-		,013		-
	N		-		41		-
p stimulated 100µmol/l	Korrelation nach Pearson		,386	,365	,413		-
	Signifikanz (2-seitig)		,012	,019	,007		-
	N		42	41	41		-

Figures

1. Study design
2. Principle of EEG mapping
3. The equipment and the staff
4. Test person at bicycle ergometer stress test
5. Test person during measurement
6. EEG-measurement
7. EEG grand average glow mode in the course of recovery with placebo
8. Average of 17 electrodes during recovery (means M1-M5): delta and theta
9. Average of 17 electrodes during recovery (means M1-M5): alpha1 and 2
10. Average of 17 electrodes during recovery (means M1-M5): beta1 and 2
11. EEG statistic map with activities in % of M1: Comparison of drink 1 vs 3
12. Mean values of hematocrite and prolactine in the course of recovery
13. Norepinephrine and epinephrine in the course of recovery
14. Dopamine and serotonine in the course of recovery
15. cyclicAMP production of MNL in the course of recovery
16. Correlations of cAMP production of MNL with EEG delta power
17. Correlations of cAMP production of MNL with EEG theta power
18. Correlations of cAMP production of MNL with serotonine
19. Correlations of cAMP production of MNL with norepinephrine

Fig.1: Schematic diagram of investigation



Measurements (M): EEG, RR, HR (continuously) and

a: blood cells, hormone levels: epinephrine, norephinephrine, dopamine, serotonine, cortisole, prolactine; blood glucose

b: cAMP

c: urine tests to find metabolites serotonine and dopamine

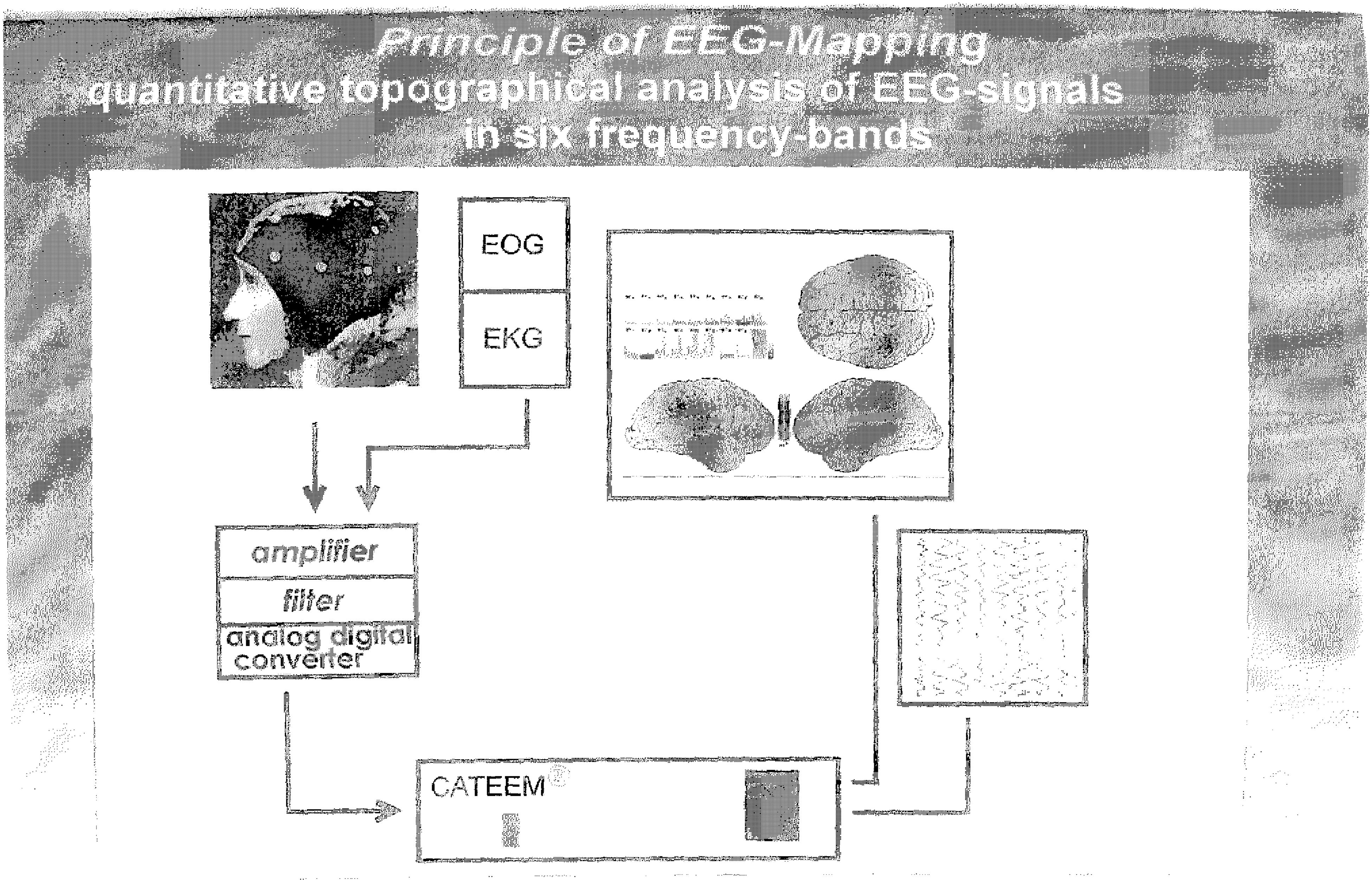

Principle of EEG-Mapping
quantitative topographical analysis of EEG-signals
in six frequency-bands
EOG
EKG
amplifier
filter
analog digital converter
CATEEM

Fig.2

The electroencephalography mapping equipment CATEEM (computer aided topographical electroencephalometry) (on the table) and the skin electrical resistance measurement Sympathicograph (before the table, one can see only a part of it) and the staff.

Fig. 3

Physical stress is induced by increasing bycicle ergometer exercise up to near maximal exhaustion. Prior to exercise the test person was prepared for measurements by electrode cap and intravenous catheter.

Fig. 4

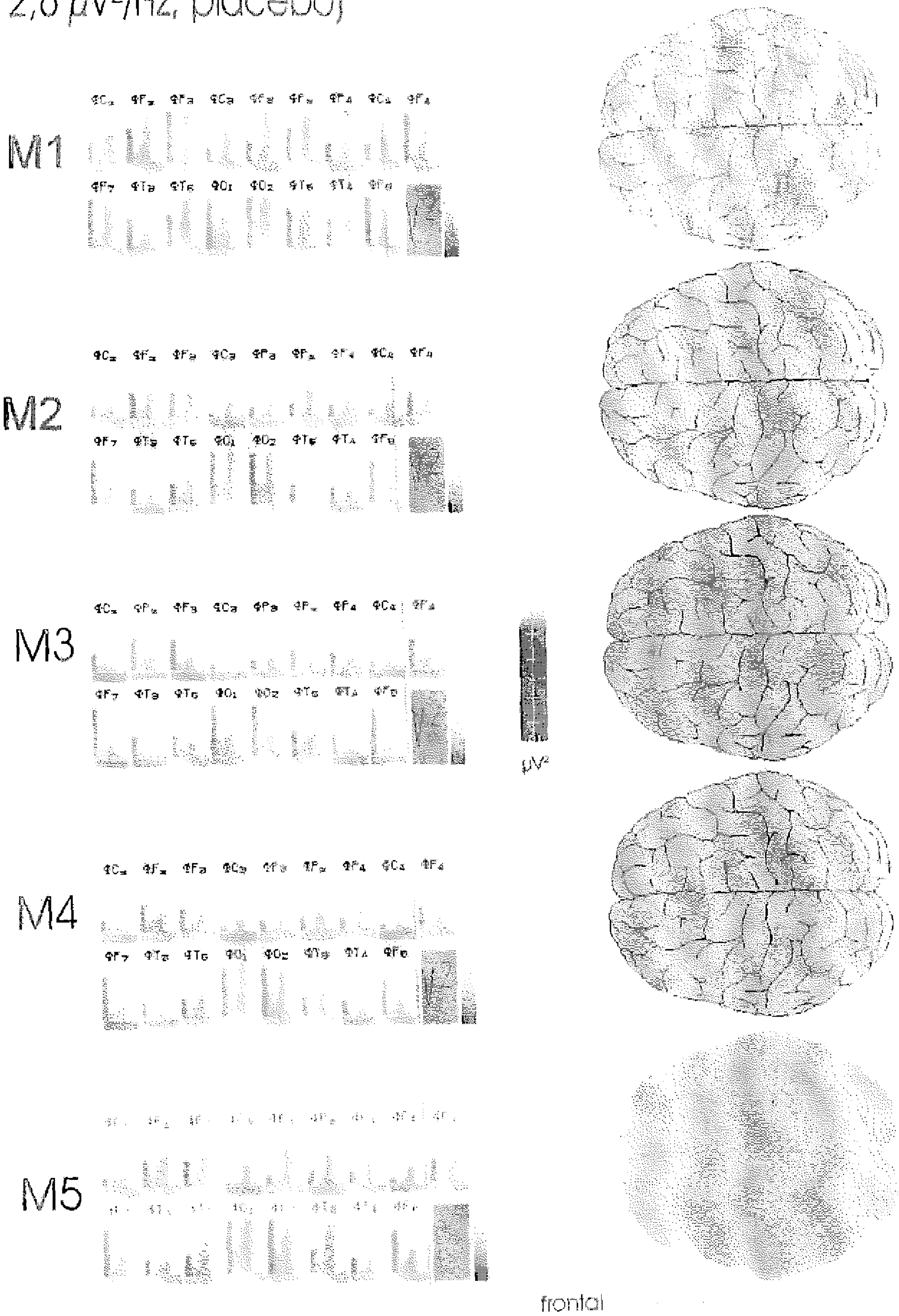
Measurements were done directly after physical stress and four times in the regeneration phase when having ingested the test drinks (fruit juice without theanine, with low or with high dosis of theanine in a randomised order), each in supine position with eyes closed, after having drawn blood from the indwelling forearm venous catheter.

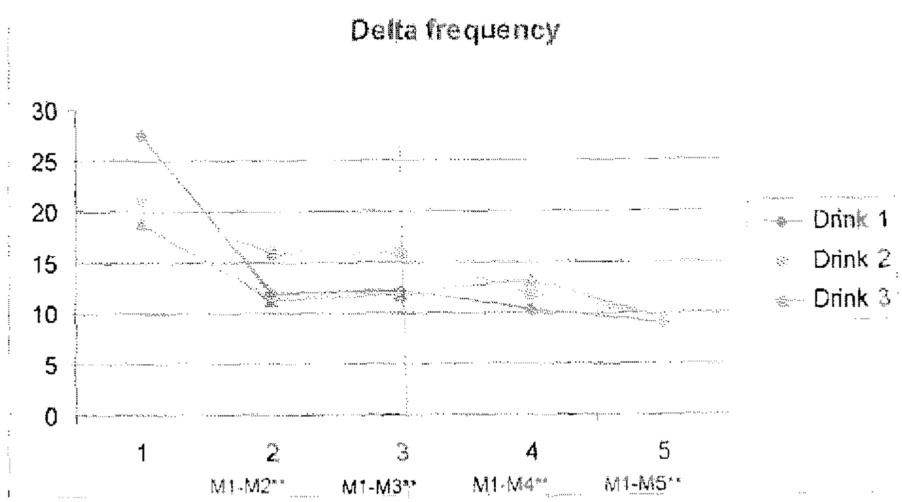
Fig. 5

The CATEEM system and the staff were in the neighbouring room, so test persons are not influenced and relaxed during the measurements. Data and maps with electrical brain activity can be seen on-line on the monitor

Fig. 6

Grand average 3 frequency map in glow mode in the course of recovery from exercise (n=14; Delta-, Alpha2-, Beta1- ; 0,5 to 2,6 $\mu V^2/Hz$, placebo)




Delta frequency
30
25
20
15
10
5
0
1
2
3
4
5
M1-M2**
M1-M3**
M1-M4**
M1-M5**
Drink 1
Drink 2
Drink 3

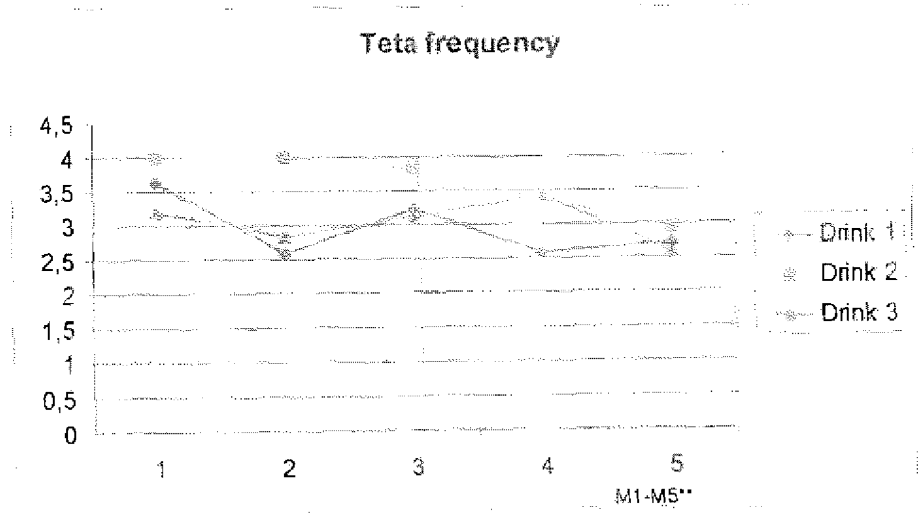
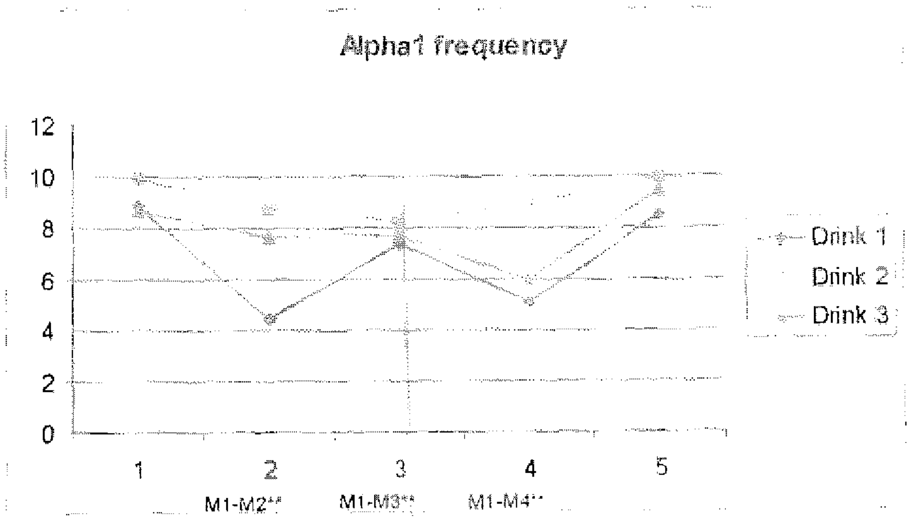

Teta frequency
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
1
2
3
4
5
M1-M5**
Drink 1
Drink 2
Drink 3

Fig. 8


Alpha1 frequency
12
10
8
6
4
2
0
1
2
3
4
5
M1-M2**
M1-M3**
M1-M4**
Drink 1
Drink 2
Drink 3

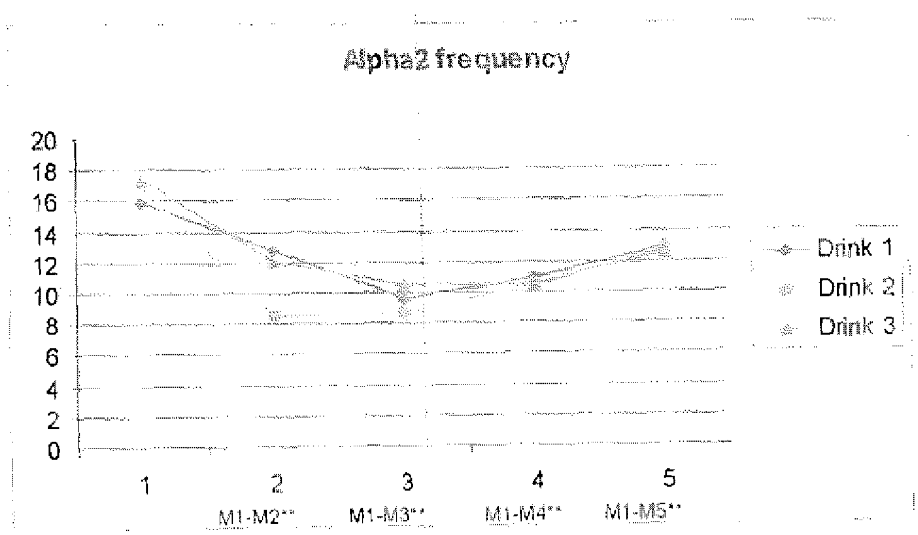
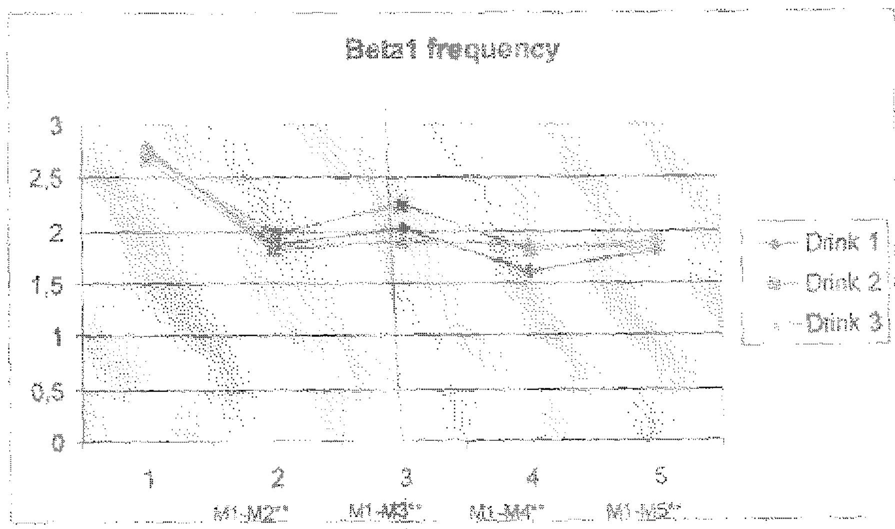
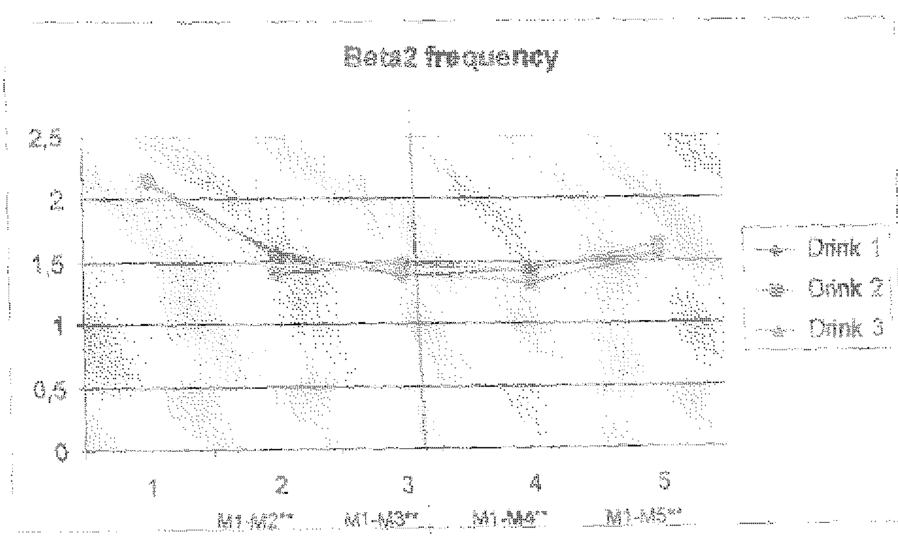

Alpha2 frequency
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
M1-M2**
M1-M3**
M1-M4**
M1-M5**
Drink 1
Drink 2
Drink 3

Fig. 9



Beta1 frequency
3
2,5
2
1,5
1
0,5
0
1
2
3
4
5
M1-M2**
M1-M3**
M1-M4**
M1-M5**
Drink 1
Drink 2
Drink 3

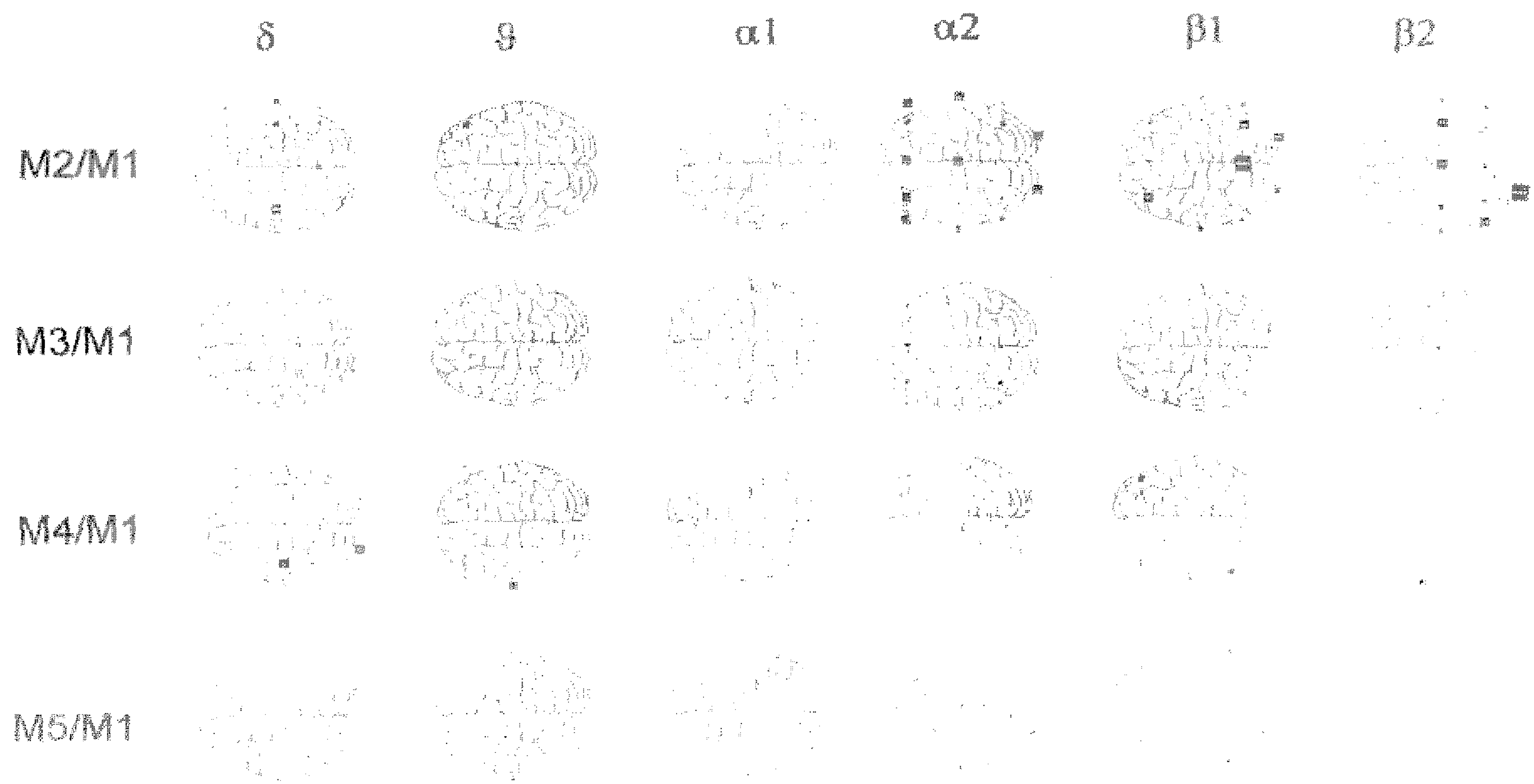


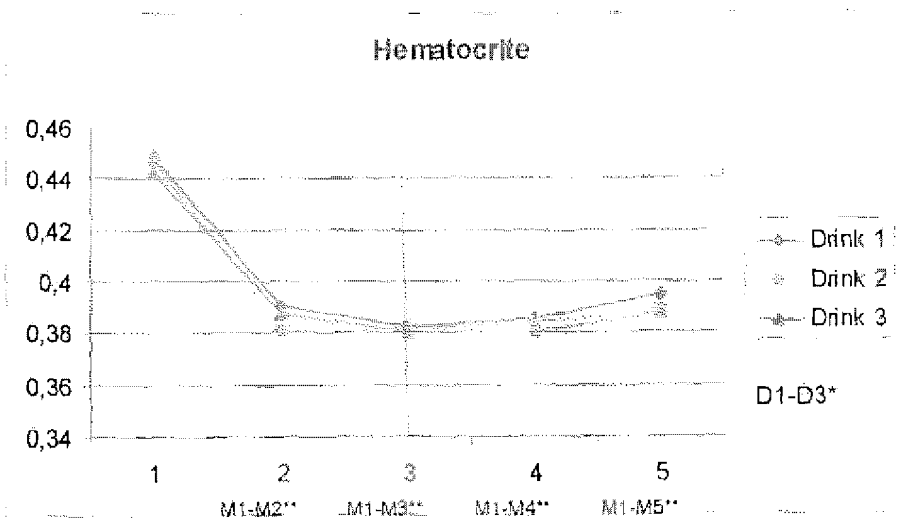
Beta2 frequency
2,5
2
1,5
1
0,5
0
1
2
3
4
5
M1-M2**
M1-M3**
M1-M4**
M1-M5**
Drink 1
Drink 2
Drink 3

Fig.11: Statistical Maps with Reference to M1 in [%]

Sign Test; N=14

Drink I vs III




Hematocrite
0,46
0,44
0,42
0,4
0,38
0,36
0,34
1
2
3
4
5
M1-M2**
M1-M3**
M1-M4**
M1-M5**
Drink 1
Drink 2
Drink 3
D1-D3*

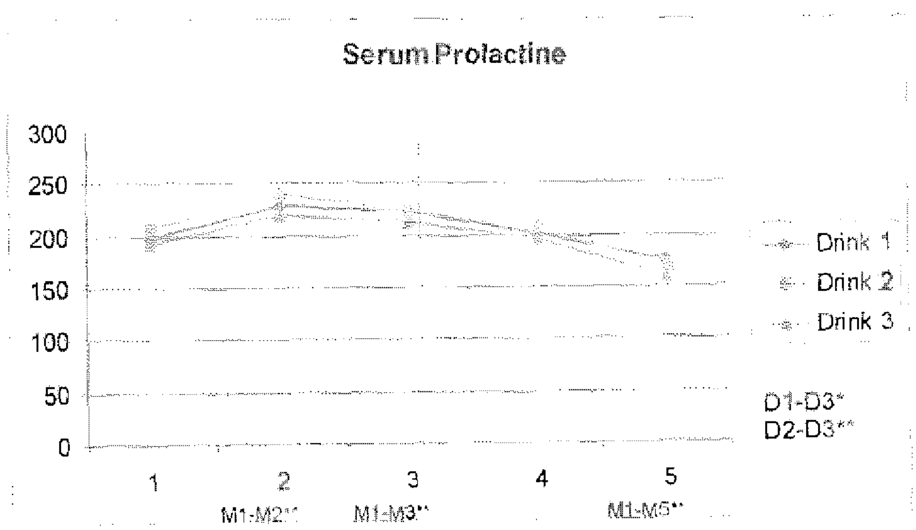
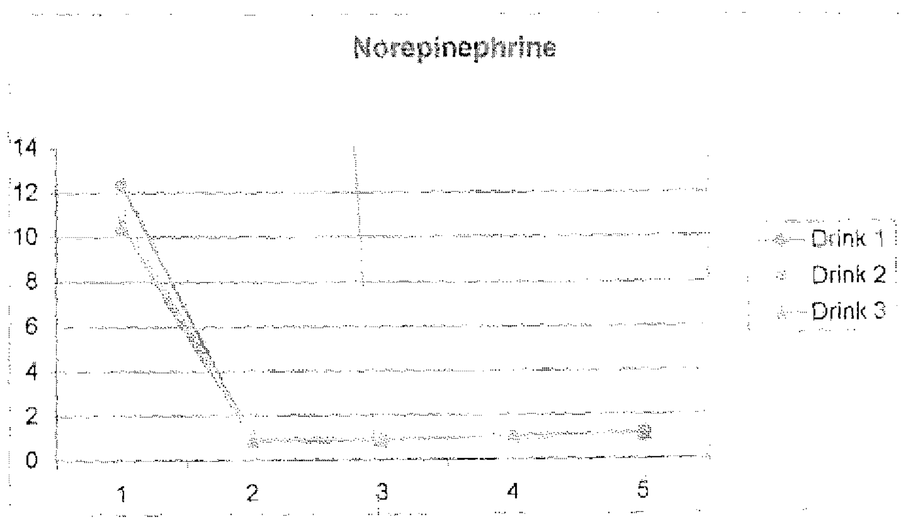

Serum Prolactine
300
250
200
150
100
50
0
1
2
3
4
5
M1-M2**
M1-M3**
M1-M5**
Drink 1
Drink 2
Drink 3
D1-D3*
D2-D3**

Fig. 12


Norepinephrine
14
12
10
8
6
4
2
0
1
2
3
4
5
Drink 1
Drink 2
Drink 3

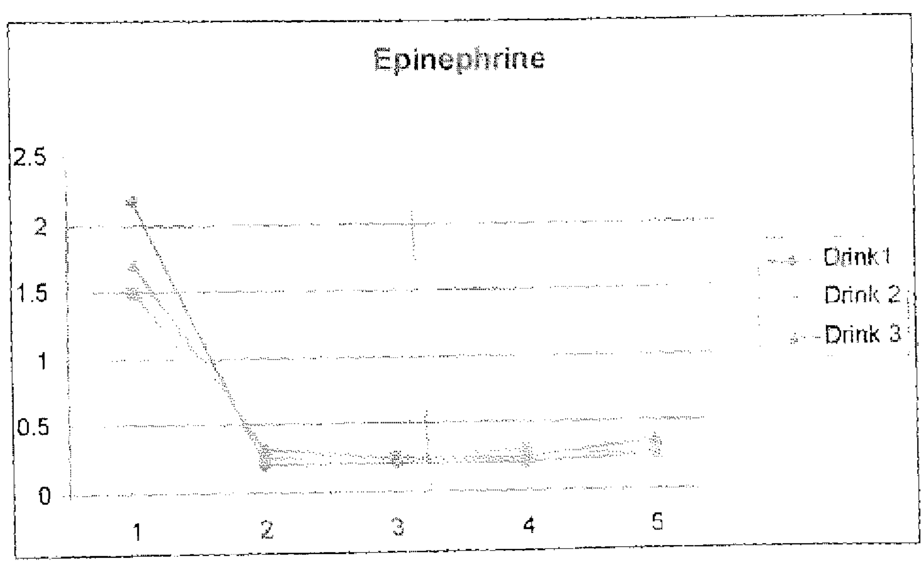
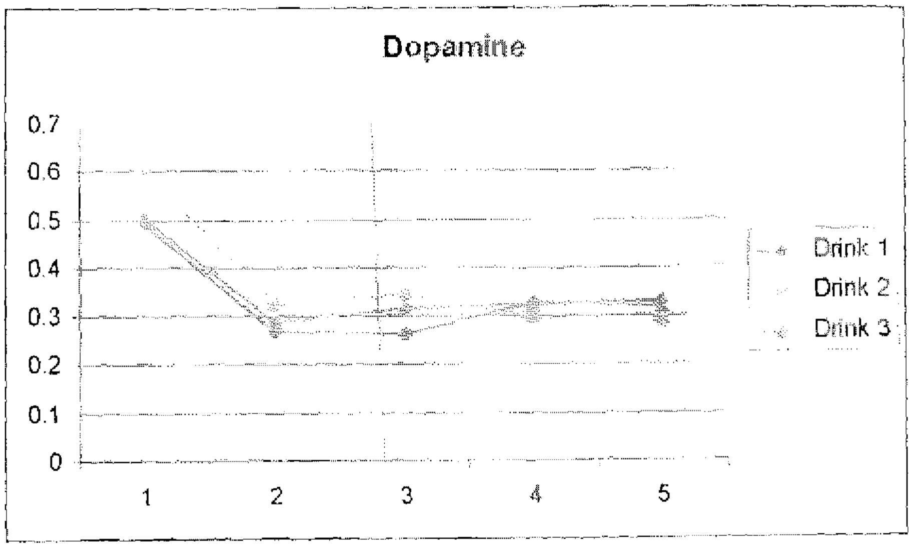

Epinephrine
2.5
2
1.5
1
0.5
0
1
2
3
4
5
Drink1
Drink 2
Drink 3

Fig. 13


Dopamine
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
Drink 1
Drink 2
Drink 3

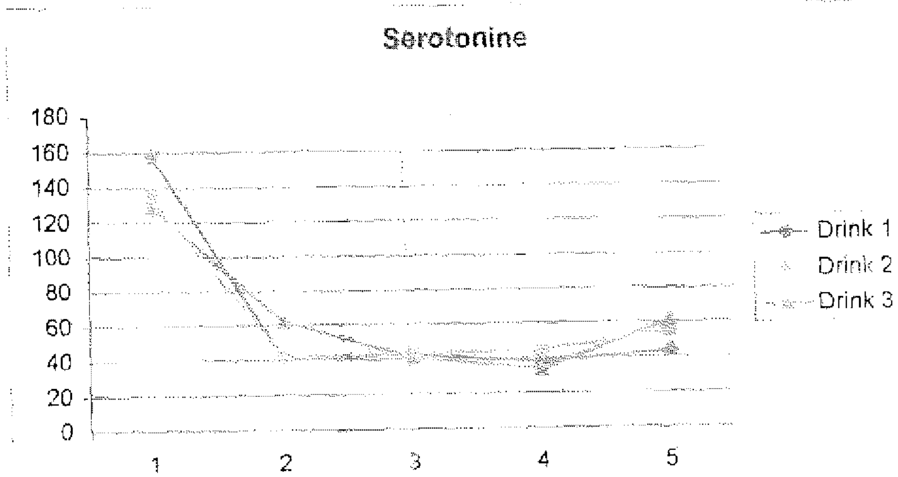
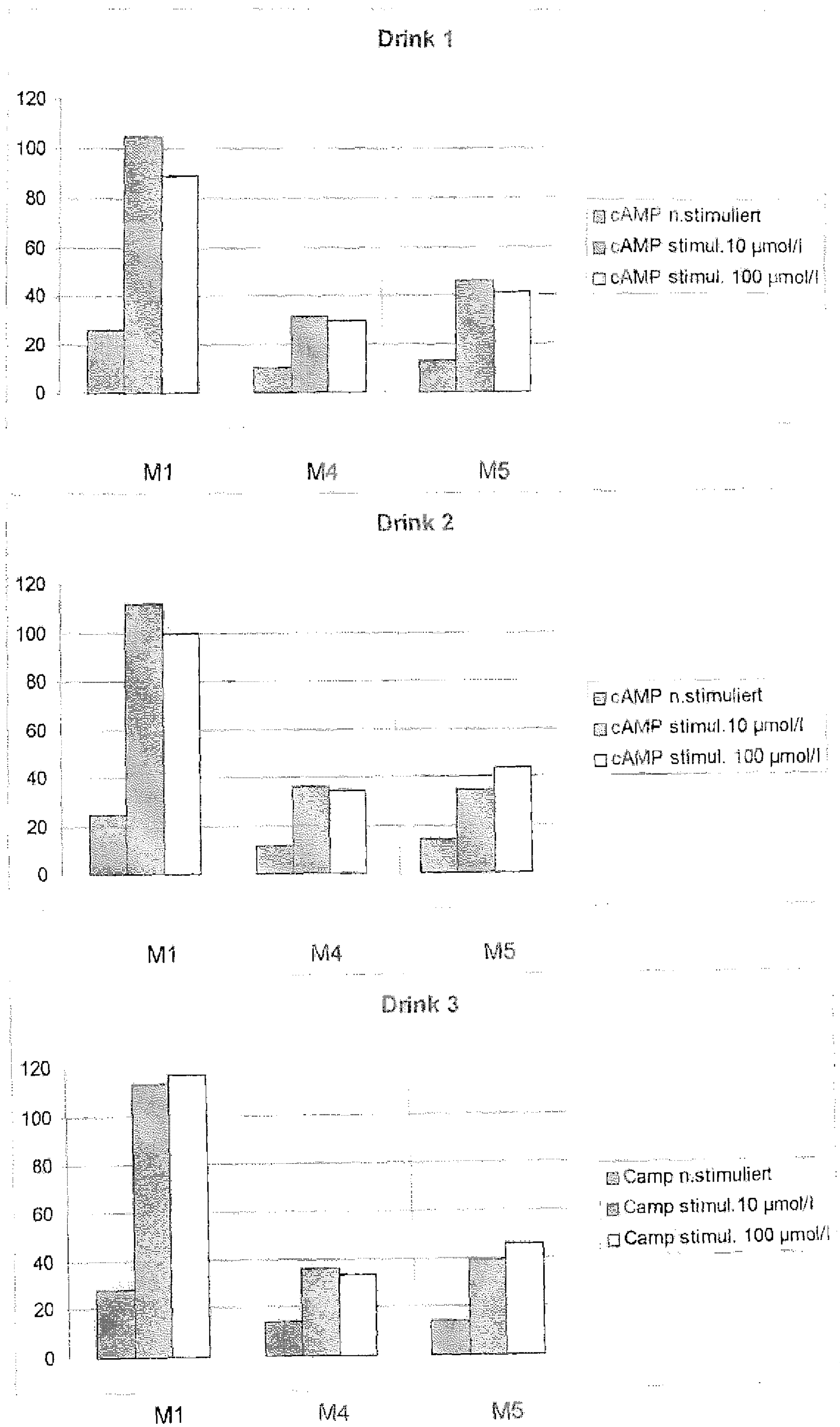

Serotonine
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
Drink 1
Drink 2
Drink 3

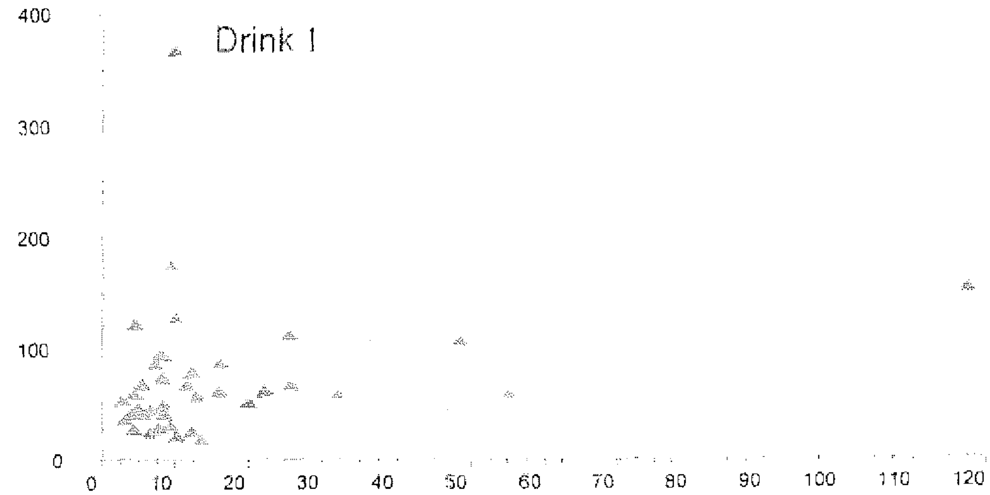
Fig. 14

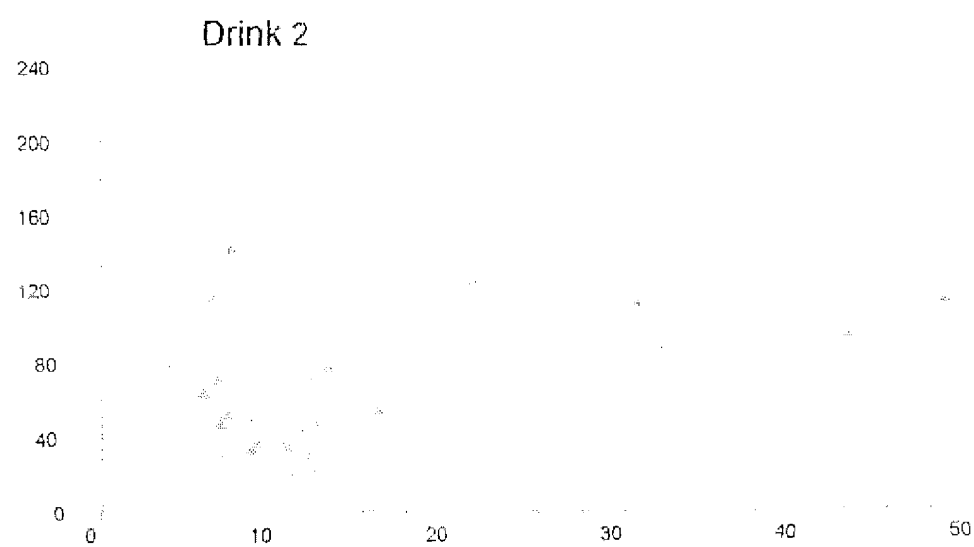


Drink 1
120
100
80
60
40
20
0
cAMP n.stimuliert
cAMP stimul.10 µmol/l
cAMP stimul. 100 µmol/l
M1
M4
M5
Drink 2
120
100
80
60
40
20
0
cAMP n.stimuliert
cAMP stimul.10 µmol/l
cAMP stimul. 100 µmol/l
M1
M4
M5
Drink 3
120
100
80
60
40
20
0
Camp n.stimuliert
Camp stimul.10 µmol/l
Camp stimul. 100 µmol/l
M1
M4
M5

Fig. 15

Delta-power/cAMP stimul. 10µmol/l





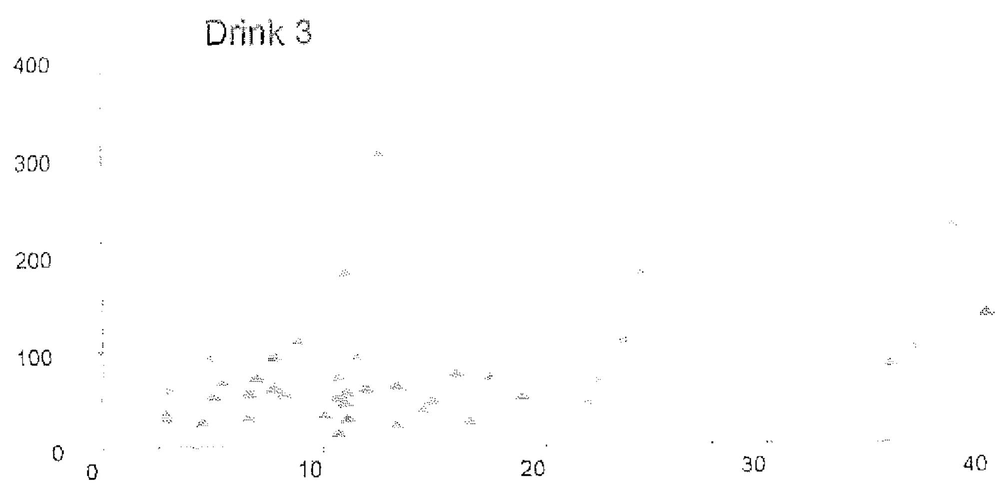


Fig. 16

Theta-power/cAMP stimul. 10µmol/l

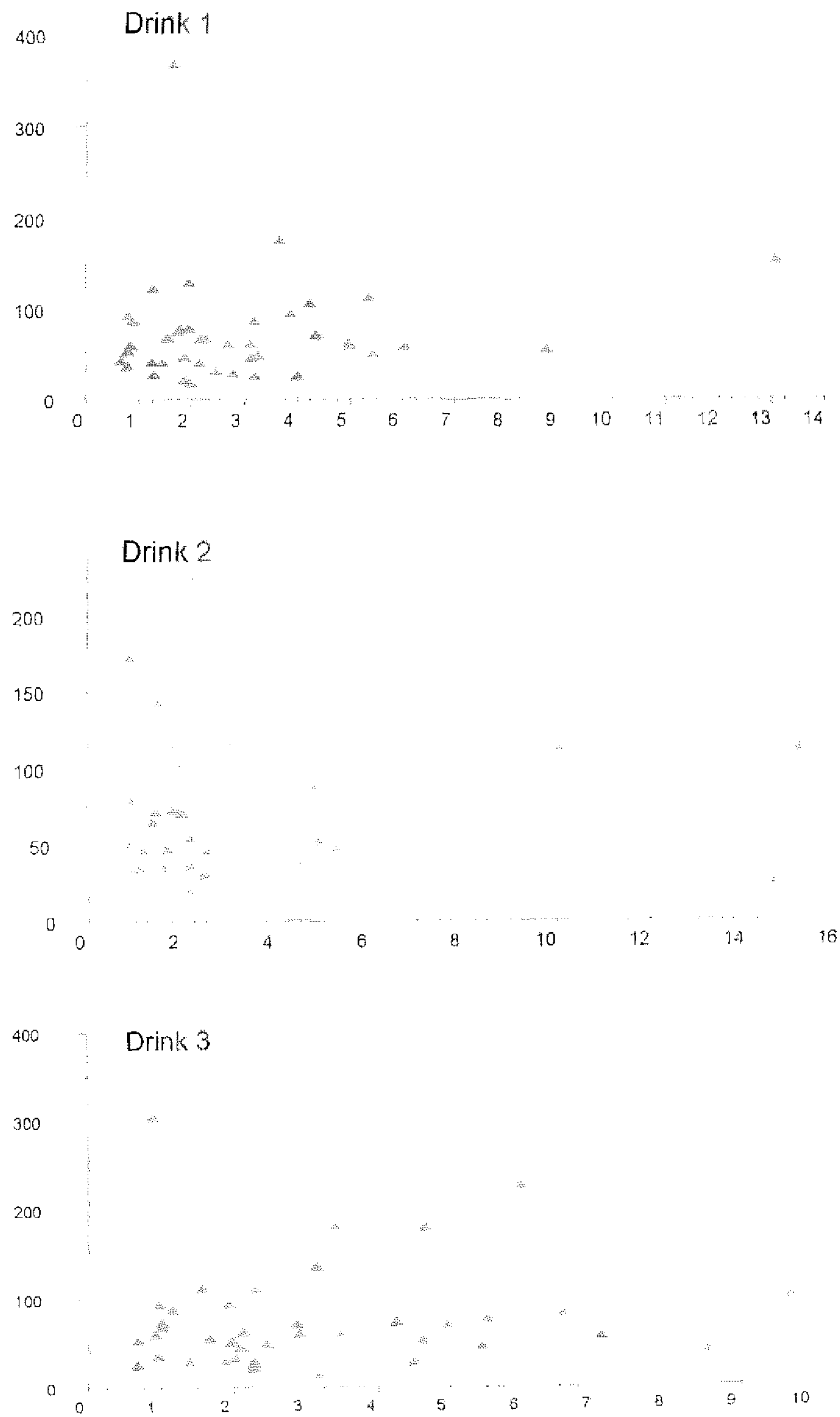


Fig. 17

Serotonine/cAMP stimul. 10μmol/l

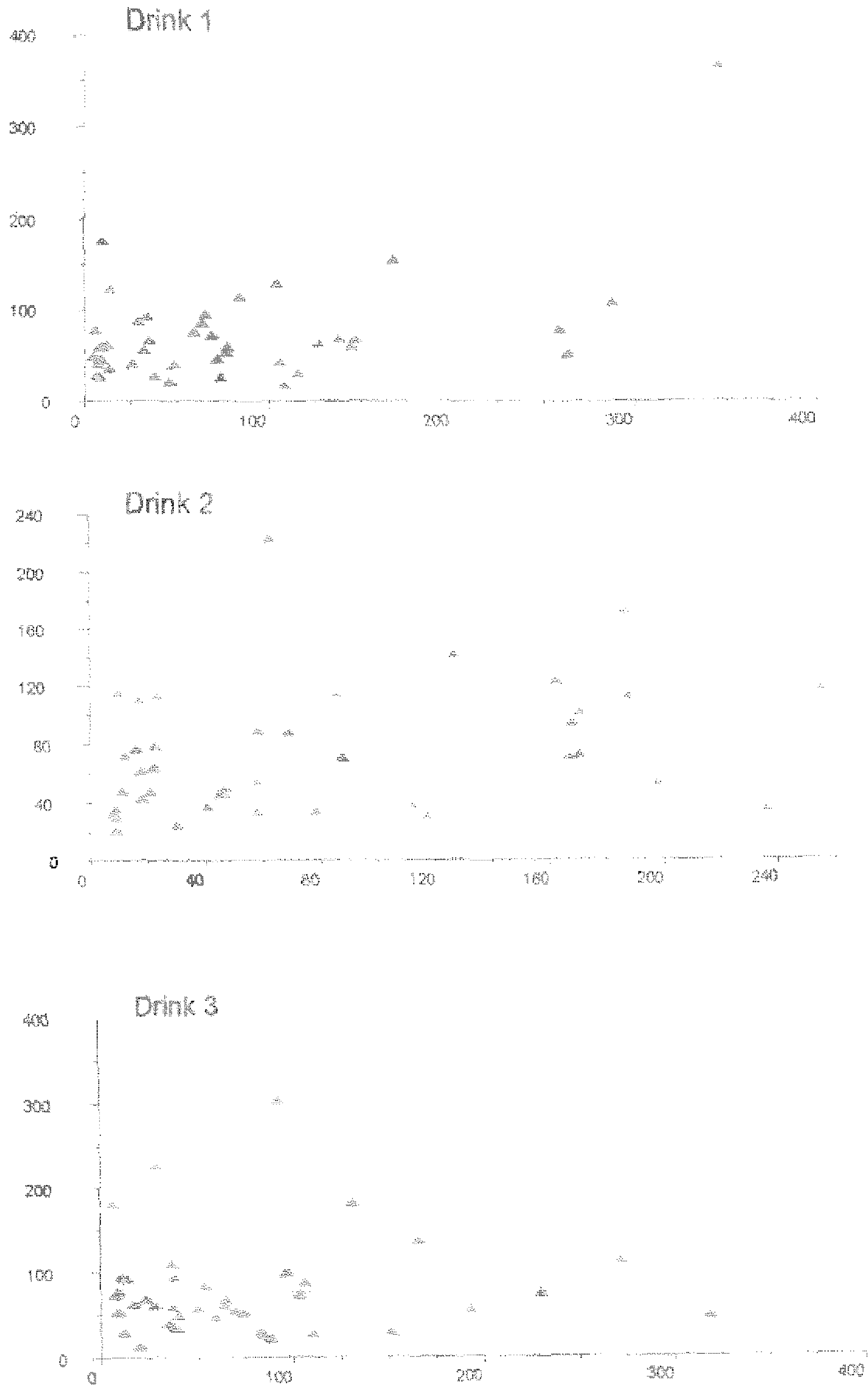


Fig. 18

Norepinephrine/cAMP stimul. 10µmol/l

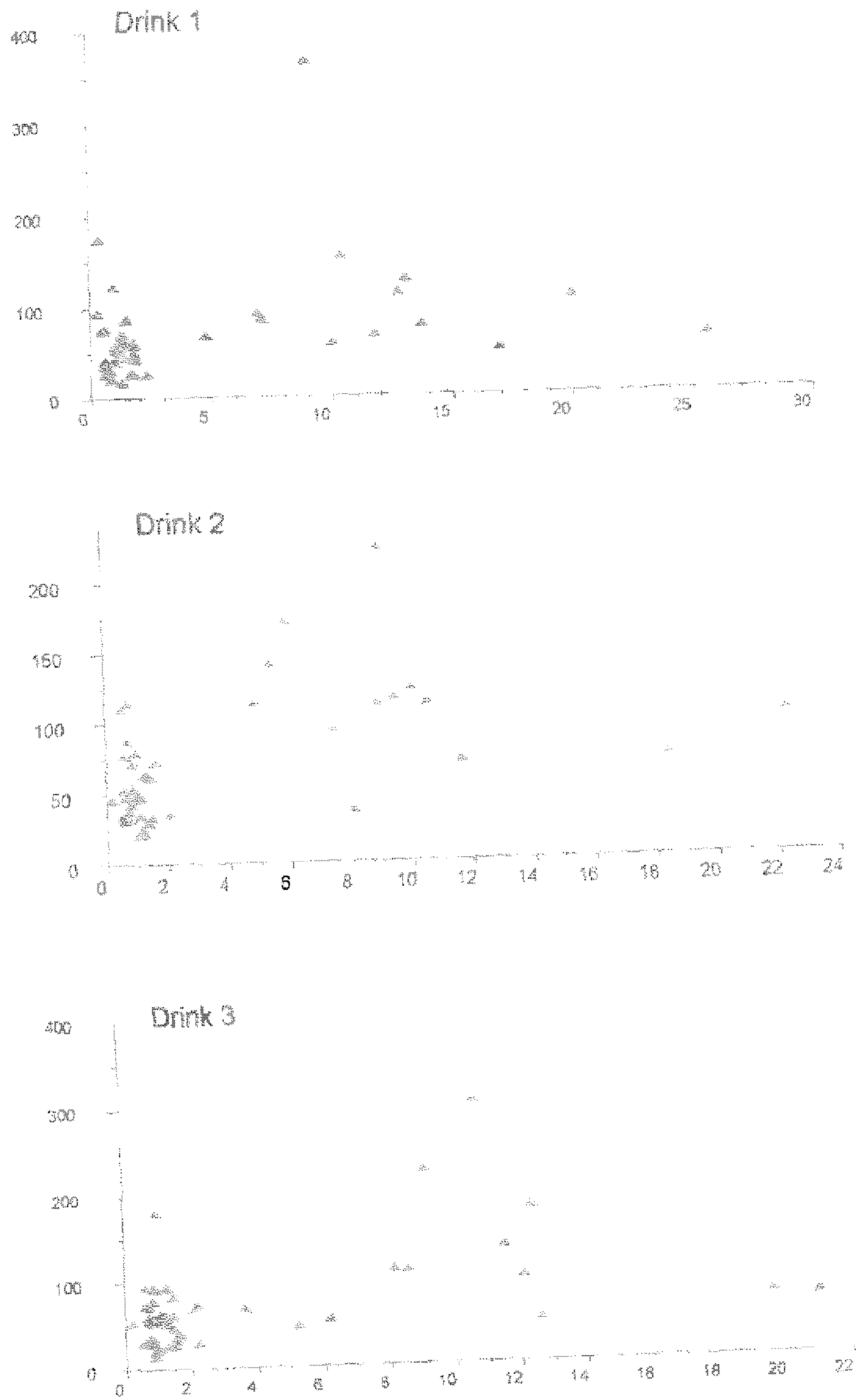


Fig.19